

# **GENETIC STOCK STRUCTURE AND ESTIMATION OF ABUNDANCE OF SWORDFISH (*XIPHIAS GLADIUS*) IN SOUTH AFRICA**

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## List of acronyms and abbreviations used

A	Number of alleles per locus
AC	Agulhas Current
AIC	Akaike Information Criterion
AMOVA	Analysis of Molecular Variance
$B_{MSY}$	Biomass that will lead to MSY
CAR	Conditional autoregressive
CCSBT	Commission for the Conservation of Southern Bluefin Tuna
CPC	Contracting party country
CPUE	Catch-per-unit-effort
CTAB	Cetyltrimethyl ammonium bromide
DIC	Deviance Information Criterion
DNA	Deoxyribonucleic acid
DWFN	Distance water fishing nation
EEZ	Exclusive economic zone
FAO	Food and Agriculture Organization of the United Nations
FCA	Factorial Component Analysis
FDR	False Discovery Rate
$F_{IS}$	Inbreeding coefficient
$F_{IT}$	Overall fixation index
$F_{MSY}$	Fishing mortality that will lead to MSY
$F_{ST}$	Fixation index
GLM	Generalised linear model
GPS	Global positioning system
$H_E$	Expected heterozygosity
HGRF	Hidden Gaussian Random Field
$H_O$	Observed heterozygosity
HWE	Hardy Weinberg equilibrium
IAM	Infinite alleles model
IATTC	Inter-American Tropical Tuna Commission
IBD	Isolation-by-distance
ICCAT	International Commission for the Conservation of Atlantic Tuna
IO	Indian Ocean
IOTC	Indian Ocean Tuna Commission
LD	Linkage disequilibrium
LJFL	Lower jaw fork length
LOA	Length overall
LOD	Log odds
LRT	Likelihood ratio test
MC	Markov Chain
MCMC	Monte Carlo Markov Chain
MLE	Maximum likelihood estimates
MSE	Management strategy evaluation
MSY	Maximum sustainable yield

mtDNA	mitochondrial DNA
$N_e$	Effective population size
PCR	Polymerase chain reaction
PIC	Polymorphic information content
RFMO	Regional Fisheries Management Organisation
$R_s$	Allelic richness
SB	Spawner biomass
$SB_{MSY}$	Spawner biomass that will lead to MSY
SEAFO	South East Atlantic Fisheries Organisation
SNP	Single nucleotide polymorphism
SSB	Spawner stock biomass
SST	Sea surface temperature
STIOG	Subtropical Indian Ocean gyre
SWIO	Southwest Indian Ocean
SWIOSG	Southwest Indian Ocean subtropical gyre
tRFMOs	Tuna Regional Fisheries Management Organisation
UN	United Nations
UNCLOS	United Nations Convention on the Law of the Sea
UNFSA	United Nations Fish Stocks Agreement
USA	United States of America
WCPFC	Western and Central Pacific Fisheries Commission

## Abstract

Targeted fishing for swordfish (*Xiphias gladius*) in South Africa began in the mid-1980s by recreational anglers. The recreational fishery dwindled with the near-shore resources at the onset of experimental pelagic longlining from 1997. The commercial fishery was formalised in 2005 with the issuing of 10-year long term rights to swordfish- and tuna-directed vessels. South Africa's swordfish catches reached a peak in 2002 at 1 187 t, and have been on the decline with average catches of 372 t over the last 5 years. South Africa straddles two ocean basins, the Indian and Atlantic Ocean and currently the jurisdictions of the Indian Ocean Tuna Commission (IOTC) and International Commission for the Conservation of Atlantic Tuna (ICCAT) are separated by a management boundary at 20°E. Consequently, all tunas and billfish stocks with the exception of the southern bluefin tuna (*Thunnus maccoyii*), are artificially divided into Atlantic and Indian Ocean stocks along this boundary, regardless of their true stock structure and distribution. Since questions remain about the origin of South African caught swordfish, it remains uncertain if the artificial split in reporting stock indices indeed reflects a biological meaningful separation of stocks.

Previous recent genetics studies have confirmed genetic differentiation between the Atlantic and Indian Ocean stocks though there is no agreement on the direction of gene flow and where, or indeed if, a genetically relevant boundary exists. Eleven microsatellite loci were included in this study of the fine scale population structure of swordfish caught relatively close inshore. Despite the poor quality of the DNA samples, muscle material of 267 swordfish caught in 2005 around the entire range of South Africa's coastline was utilised. A posterior predictive map of admixture proportions produced a potential admixture zone between 14°E and 27°E. There is evidence of gene flow and migration in this area in both directions, though the evidence for weak differentiation suggests that the Indian Ocean and Atlantic Ocean contain separate stocks and that swordfish stocks coexist around South Africa but return to their ocean of origin to reproduce. Due to passive drift of larvae and active dispersal of adults that have wide environmental parameter limits that extend across this area, swordfish would be prone to admixture and genetic homogenisation.

Catch rate or CPUE is under certain circumstances assumed to be proportional to abundance. Standardised catch-per-unit-effort (CPUE) indices were obtained with a generalised linear model (GLM) of swordfish catch and effort data from the South African pelagic longline fleet operating in the South Atlantic and Indian Oceans between 1998 and 2014. The tweedie model with a Poisson distribution and a log-link function was chosen to model the catch. The factors included in the model were season, sea surface temperature (SST), vessel length overall (LOA), fishing tactic, latitude,

longitude and three interaction terms, LOA\*latitude, LOA\*longitude and season\*SST. Concerning the genetically determined admixture zone between 14°E and 27°E, comparisons were made between models that included, and excluded, these data in the South Atlantic Ocean (<20°E) and the Indian Ocean (≥20°E). Total deviance explained by the models that excluded the admixed data were 49.21% and 61.95% for the South Atlantic and Indian Ocean, respectively, of which year & season and year & LOA explained a majority of the variation. All of the standardised CPUE trends experienced a peak at the start of the fishery and have been undergoing a steady decline since. There were no qualitative differences in the abundance trends of the models that included the admixed zone with those that did not. Therefore, the inclusion of abundance indices from South Africa should not be discarded on account of stock origin concerns in this area.

Based on the population genetics results, the ICCAT South Atlantic quota allocated to South Africa is currently fulfilled with swordfish that originate from two different stocks. Accounting for this admixture zone has implications for (i) how South Africa's data is included in stock assessments, (ii) how the total catch is reported and (iii) how quotas are managed between ICCAT and IOTC, particularly when quotas and the status of the stocks differ vastly. The suggested management measures for swordfish would be:

- (i) Include South Africa's abundance indices data in the stock assessments at the current 20°E boundary for the South Atlantic (<20°E) and the Indian Ocean (>20°E) stocks.
- (ii) South Africa report catch and effort data for this transitional region (14°E – 27°E) to both tRFMOs (*i.e.* duplicate data will be held by ICCAT and IOTC for this region).
- (iii) The tRFMOs consider that 50% of the catch in this transitional region is swordfish that has originated from their respective stocks.

The tRFMOs are progressively implementing the Management Strategy Evaluation (MSE) approach to meet their objectives. Further studies on spatial-temporal variations in the dynamics of swordfish (and other migratory stocks) population mixture between the Indian and Atlantic Oceans has the potential to be incorporated in the MSE framework for ICCAT and IOTC.



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## CHAPTER 1

### History and review of commercial swordfish (*Xiphias gladius*) catches in South Africa

#### *Biology*

Swordfish *Xiphias gladius* (Linnaeus 1758, *Xiphias* from Ancient Greek meaning “sword” and *gladius* from the Latin word “sword”, the only surviving species of the family Xiphiidae) is a cosmopolitan, oceanic, migratory species that has an elongated, robust body and a long flat upper jaw that extends to resemble a bill. All teeth and scales are lost by adulthood (Saxton, 2012). Unlike tuna, which have mostly 'red' muscle which is good for endurance activities, the mostly 'white' muscle of swordfish is not suited to swimming for long periods without fatigue (Ward and Elscot, 2000). Therefore, swordfish undertake long migrations by moving with prevailing currents (Ward and Elscot, 2000).

Swordfish occur in all temperate and tropical oceans from approximately 60°N to 45°S. They have been found to move between tropical spawning and temperate feeding grounds (Neilson *et al.*, 2009) and prefer sea surface temperatures (SST) of 18°C to 22°C, with larvae and juveniles rarely found below 24°C. Swordfish, however, have one of the widest temperature tolerances compared to other tuna and billfish with a range of between 10°C and 28°C (Sedberry and Loefer, 2001). Determining the age of swordfish is difficult since the otoliths are very small and delicate and scales are missing in adults (Beamish, 1981).

Swordfish are opportunistic predators and will have a variety of prey species in their diet, though cephalopods are preferred (Young *et al.*, 2006). Although it is not a gregarious species, high concentrations of this species can be found associated with oceanographic features that create turbulence and sharp temperature breaks and which concentrate prey, such as mesoscale eddies, (Kai and Marsac, 2010).

The maximum reported size of swordfish caught has been 445 cm LJFL and 540 kg. All fish larger than 140 kg are usually females (Nakamura, 198526). The males are faster growing and reach maturity a year earlier than females (Taylor and Murphy, 1992). There are discrepancies in studies on the size at first maturity of females (LJFL<sub>50</sub>), due largely to the methods used and the sampling areas-times and groups of fish selected (Mejuto and García-Cortés, 2014). A recent study by Mejuto and García-Cortés (2014) that sampled 18 279 females in regions exhibiting a considerable amount of maturation-reproductive activity, estimated the size at first maturity of females (LJFL<sub>50</sub>) at 146.5 cm LJFL.

Known spawning grounds are located in the tropical regions- the Caribbean and Gulf of Mexico in the Atlantic Ocean; central north, south west and equatorial regions in the Pacific; the Mediterranean and south Madagascar and La Reunion Island in the Indian Ocean (Taylor and Murphy, 1992; Poisson and Fauvel, 2009; Neilson *et al.*, 2013). Swordfish are batch spawners with the spawning season varying per region (Neilson *et al.*, 2013). Swordfish larvae tend to drift with currents (Reed and Cannon, 2009). Research and habitat use and movement of adult swordfish have been at the forefront to mitigate the losses through spatial management of fishing effort (Rooker *et al.*, 2012). In contrast, the understanding of habitat use during the critical early life period is far less studied even though larval indices are valuable for identifying spawning/nursery grounds (Richardson *et al.*, 2009) and assessing population trends (Rooker *et al.*, 2012).

Tagging studies have revealed extensive horizontal movements of swordfish, swimming thousands of kilometres (Neilson *et al.*, 2014). Previous tagging studies have reported seasonal cyclic horizontal movement of swordfish, related to temperature, food availability and spawning. Takahashi *et al.* (2003) hypothesized a cyclic migration of swordfish in the Northwest Pacific between food-rich, cold-current areas during summer and the subtropical wintering area. The results from Neilson *et al.* (2009) of swordfish tagged in Canadian waters also demonstrated a consistent pattern of movement with residence in temperate waters from June to October, followed by migration to the south into the Caribbean Sea, with fish remaining there until April. In the Southwest Pacific, Abascal *et al.* (2010) found tagged swordfish to move northwest as the water temperature decreased and to return south at the onset of Spring, postulated to be related to food availability. Similarly, swordfish in the North Atlantic displayed seasonal patterns, with fish generally moving south by the end of the year and returning to the temperate foraging grounds in spring (Abascal *et al.*, 2015). Electronic tagging is a tool that can indicate the dynamics within stock and between adjacent stocks. The stock structure and movement patterns of swordfish studied by Evans *et al.* (2012) with electronic tagging have provided evidence for revising spatial assumptions used in stock assessments.

Extensive diel vertical migration patterns of swordfish, predominantly shallow at night and deep diving during the day, are in association with the deep scattering layer and the movement of mesopelagic organisms (Carey, 1990). The depth range for this species is from the 0 – 30 m to depths of around 1100 m (Abascal *et al.*, 2010). Swordfish have a unique circulatory mechanism where the muscle and brown tissue warms blood flowing to the eyes and brain, an adaptation for predation at depth (Carey, 1982). Basking swordfish can be seen at the surface during the day in some areas (e.g. Dewar *et al.*, 2011).

### *Early fishing for swordfish*

Fishing for swordfish dates back to ancient times in the Mediterranean Sea from around 1000 BC when the Romans and Greeks had a harpoon fishery in the Straits of Messina (Ward and Elscot, 2000). Established commercial fisheries began, centuries earlier as near-shore subsistence operations involving rods, harpooning or driftnets to catch swordfish in countries as such as the United States of America (U.S.A.), Canada (Tibbo *et al.*, 1961; Fisheries and Oceans Canada, 2009), Chile (Jenkins, 1988). In the 1800s, commercial fishermen in New England were pursuing swordfish, primarily with harpoons to target large swordfish in the surface waters. Japan is reportedly the first country to begin longline fishing during the Meiji Era (1868-1912) and the fleet began to fish further offshore from the 1920s (Barclay and Koh, 2005). From the 1930s onwards other countries started to use longline equipment to catch swordfish. By the 1980s Japan, Spain, Chinese Taiwan, Italy, U.S.A. and Chile were the top swordfish catching countries (Govender *et al.*, 2003). Between 1986 and 1997 these six countries, together, caught 77% of the world's swordfish catch in the following proportions: Japan (23%), Spain (19%), Chinese Taiwan (12%), Italy (10%), U.S.A. (8%) and Chile (5%) (Govender *et al.*, 2003).

The two main markets for swordfish are in Europe and the U.S.A., with a smaller demand in South East Asian, where swordfish is consumed as steaks and less frequently as sushi. Although swordfish is regarded as a high-value catch, it is largely caught as bycatch in longlining operations that target tuna. Market prices affect the level of fishing pressure on swordfish resources, whether targeted or bycatch (Ward, 2000; Miyake, 2006).

The earliest reported sighting and capture of swordfish in South Africa was in 1777 by William Patterson in his book *A Narrative of Four Journeys into the country of the Hottentots and Caffaria, from the years 1777 to 1779* (Horne, 1959). He noted the presence of a prodigious number of swordfish and porpoises in Table Bay, though this claim is unconfirmed (Horne, 1959). The first authentic record of a swordfish caught in South Africa was in 1956 from the trawl vessel *George Irvin* (Horne, 1959), a 2.74 m and 59.1 kg gutted weight specimen caught 40 miles west of Slangkop. In 1958, swordfish caught 15 miles south west of Cape Point, a 1 m and 18 kg whole weight specimen, constituted the first record of a rod and reel catch of this species in South Africa (Horne, 1959).

Over the following 16 years, swordfish were caught occasionally by the trawlers or sighted but unsuccessfully targeted by game fishermen off Cape Point (Smal, 2014). Swordfish were challenging to bait and hook. Although there were few records of swordfish catches, this was not an indication of lack of abundance. Game fishing for swordfish struggled to take off as only a few persistent fishermen claimed success (Smal, 2014). It was speculated then that “South Africa is not a swordfish area” and the only swordfish likely to be caught were the ones surrounding the Cape on their migration (Horne, 1959). Interest sparked amongst the recreational angling community in the mid-1980s when swordfish were targeted and successfully landed (Smal, 2014). Around the same time, the International Commission for the Conservation of Atlantic Tuna (ICCAT) had implemented swordfish catch restrictions in the North Atlantic, prompting interest in swordfish availability in the South Atlantic (Govender *et al.*, 2003). Attempts were made to protect the resource around the Cape from the longline vessels, but at the onset of local longlining in 1997 the recreational fishery for swordfish dwindled with the near-shore resource.

Commercial fishing for large pelagic species in South Africa dates back to the 1960s (Welsh, 1968 and Nepgen, 1970). Exploitation of large pelagic species in South Africa can be divided into four sectors, 1) pelagic longline, 2) tuna pole-line 3) commercial linefishing (rod and reel) and 4) recreational linefishing. Pelagic longline vessels are the only vessels that target swordfish, with some bycatch being caught by mid-water trawl vessels. South African swordfish do not display basking behaviour (West *et al.*, 2012) and hence does not support a harpoon fishery; and gillnet fishing in South Africa is prohibited. Pelagic longline fishing by South African vessels began in the 1960s with the main target being southern bluefin tuna (*Thunnus maccoyii*) and albacore (*Thunnus alalunga*) (Welsh, 1968 and Nepgen, 1970). This South African fishery ceased to exist after the mid 1960's, as a result of a poor market for low quality southern bluefin and albacore (Welsh, 1968). However, foreign vessels, mainly from Japan and Chinese-Taipei, continued to fish in South African waters from the 1970s until 2002 under a series of bilateral agreements. Interest in pelagic longline fishing re-emerged in 1995 when a joint venture with a Japanese vessel confirmed that tuna and swordfish could be profitably exploited within South Africa's waters. The last comprehensive report on the developments of the pelagic longline fishery in South Africa was prepared by Penney and Griffiths (1999) during the experimental phase of the fishery. Thirty experimental longline permits were issued in 1997 to target tuna, though substantial catches of swordfish were made during that period (Penney and Griffiths, 1999). In 2005 long term (10 year) rights were issued to 18 swordfish-directed vessels and 26 tuna-directed vessels. Fewer rights were issued towards swordfish-directed targeting to reduce the effort and subsequent catch of the rapidly declining resource (West and

Kerwath, unpublished). Although swordfish is at its southern limits in South Africa's exclusive economic zone (EEZ), catches at the beginning of the fishery were substantial enough to warrant a dedicated targeted fishery (Figure 1).

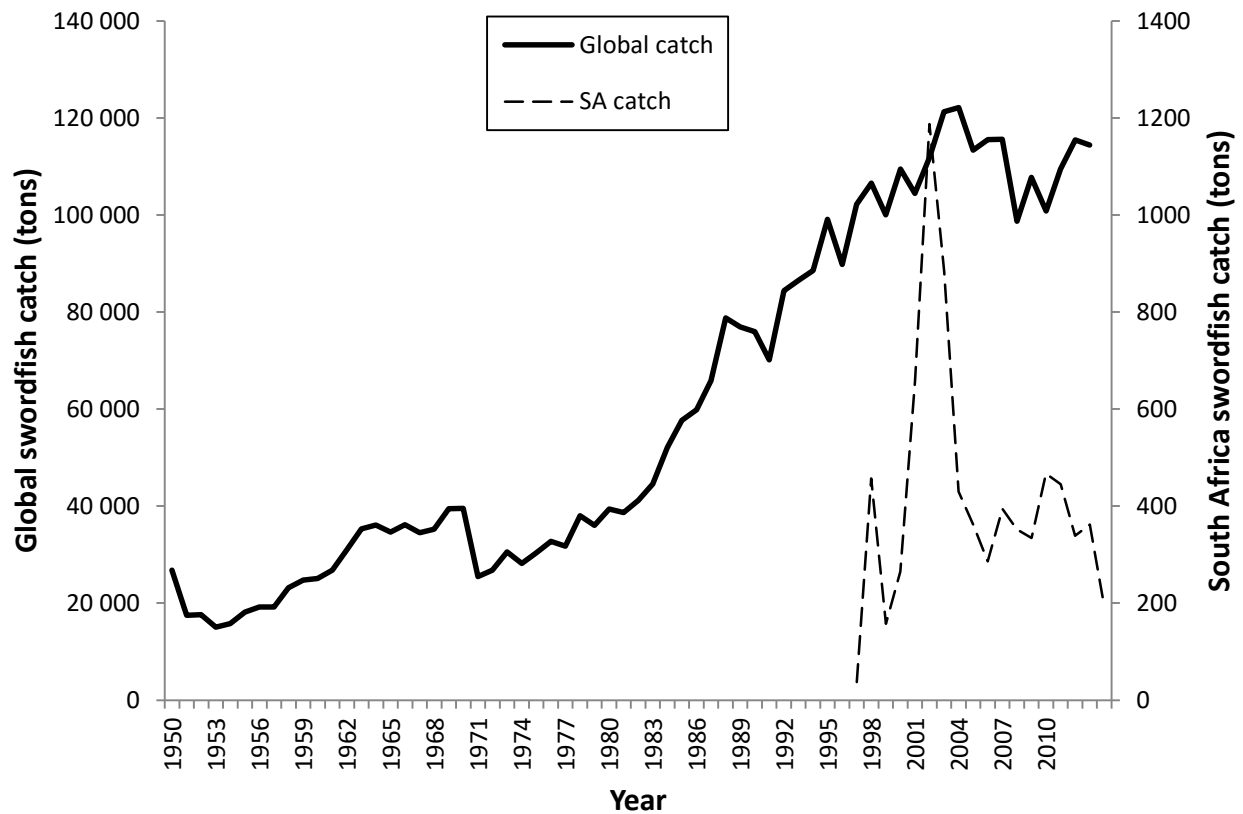


Figure 1. Global swordfish catches (in tons) from 1950 – 2013 (©FAO, 2016) and local swordfish caught by the South African pelagic longline fleet from 1997 – 2014.

### Fishing methods

In the Indian and Atlantic Oceans, swordfish are caught by harpoon (*e.g.* Canada), drifting longline (*e.g.* Canada, Spain, Portugal, Japan, China, Chinese-Taipei, Morocco, Uruguay, U.S.A., Brazil, France) and artisanal drifting gillnets (*e.g.* Iran, Pakistan, Sri Lanka). Pelagic longline gear can be classified into types of mainline that target swordfish, American-style monofilament and Spanish-style multifilament (Mejuto and De la Serna, 2000; Pons *et al.*, 2014). Swordfish are generally targeted at night in the surface layers, usually around 50 m depth (Mejuto *et al.*, 2013). Japanese-style of longlining with multifilament targets mainly bigeye (*Thunnus obesus*) and yellowfin tuna (*Thunnus albacares*) with deeper sets at around 300 m. The vessels that catch swordfish vary between freezer and fresh (ice vessels), depending on the market destination, and whether the swordfish are targeted or bycatch.

The local South African vessels are on average smaller (~25 m) than the foreign-flagged vessels and have traditionally targeted swordfish with the American-style monofilament gear. The foreign-flagged vessels fishing in South Africa have on average larger vessels (~45 m) and as such are able to fish further offshore; targeting yellowfin and bigeye tunas seasonally in the Indian Ocean. The local South African vessels utilise 20 m long buoylines and 20 m long trace lines/branch lines. The trace lines consist of a 16 m leader connected by a lead swivel to 4 m hook trace of 2 mm monofilament nylon to the hook that ranges in size from 14/0 to 18/0. The lines are typically deployed in a single, night set in each 24 hour period, and hauled the following day. Hooks are baited with squid and lightsticks and clipped to the main line during the set. The length of the line deployed averages between 35 nautical miles (nm) and 40 nm per night, and the number of hooks ranges from about 800 – 1 000 per set (Penney and Griffiths, 1999).

#### *Swordfish catch trends*

From 1950 the global catch of swordfish increased steadily until the sharp increase in the 1980s, which was likely due to the emergence of longline fleets capable of fishing further offshore (©FAO, 2016) (Figure 1). Globally, the countries that caught the most swordfish in 2013 were Spain, Chinese-Taipei and Japan (Table 2) (©FAO, 2016). Swordfish catches have been dominated by longline fleets either catching swordfish as target or bycatch. The countries with the highest longline-caught swordfish catches in the South Atlantic Ocean in 2013 were Spain, Brazil and Japan (ICCAT, 2015) (Table 2). In the Indian Ocean, Chinese-Taipei and Spain, both using exclusively longline gear, were the top performers along with Sri Lanka that uses longline, gillnet and handline gear to catch swordfish (IOTC, 2015) (Table 2). In recent years, the prevalence of piracy in the Indian Ocean has reduced the fishing effort there, displacing vessels opting to neighbouring oceans (IOTC, 2013).



Table 2. The fleets with the largest global, South Atlantic and Indian Ocean swordfish catch (in tons) in 2013.

Global		South Atlantic		Indian Ocean	
Spain	29502	Spain	4184	Chinese Taipei	5686
Chinese Taipei	12944	Brazil	1312	Sri Lanka	5537
Japan	10163	Japan	684	Spain	4767
Indonesia	5989	Chinese Taipei	582	Indonesia	3892
Sri Lanka	5537	Peoples Republic of China	196	Indian	1926
Chile	4852	Senegal	178	Seychelles	1175
Philippines	4177	South Africa	171	Portugal	1085
China	3416	Belize	136	Iran	804
United States of America	3272	Namibia	129	Reunion Island	785
Italy	2863	Portugal	125	Pakistan	689
Portugal	2833	Côte D'Ivoire	110	Tanzania	669

South Africa's swordfish catches reached a peak in 2002 at 1 187 t, and have been on the decline ever since (Figure 1). The average catch over the last 5 years is 372 t (Figure 1). Swordfish of between 120 and 220 cm LJFL are commonly caught (Figure 2). The fishing areas for swordfish have been from the Agulhas Bank region along the entire West Coast, and off Richards Bay towards the Mozambique Channel (Figure 3). Swordfish-directed vessels are increasingly changing their target focus towards tunas for better catch returns.

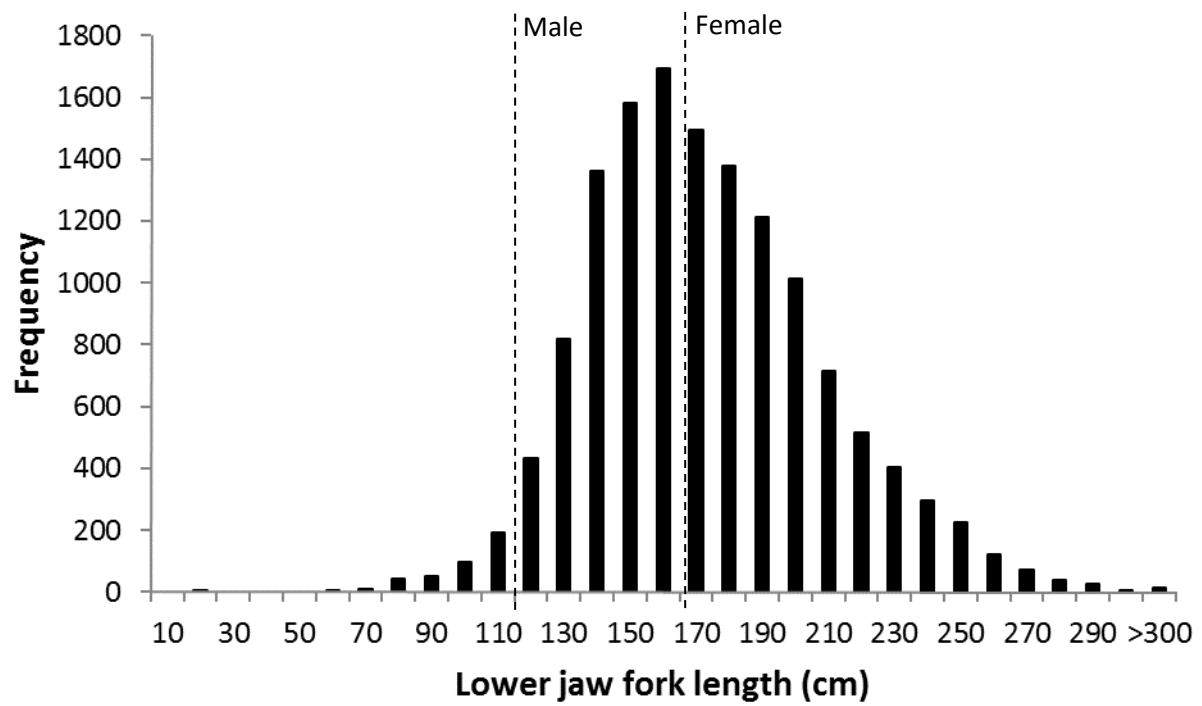


Figure 2. The length frequency of swordfish caught in the pelagic longline fishery from 2002 – 2012 in South Africa. Dashed lines denote sizes at 50% maturity for males (119.8 cm  $\pm$  1.9 cm LJFL) and females (170.4 cm  $\pm$  2.4 cm LJFL) (Poisson *et al.*, 2001).

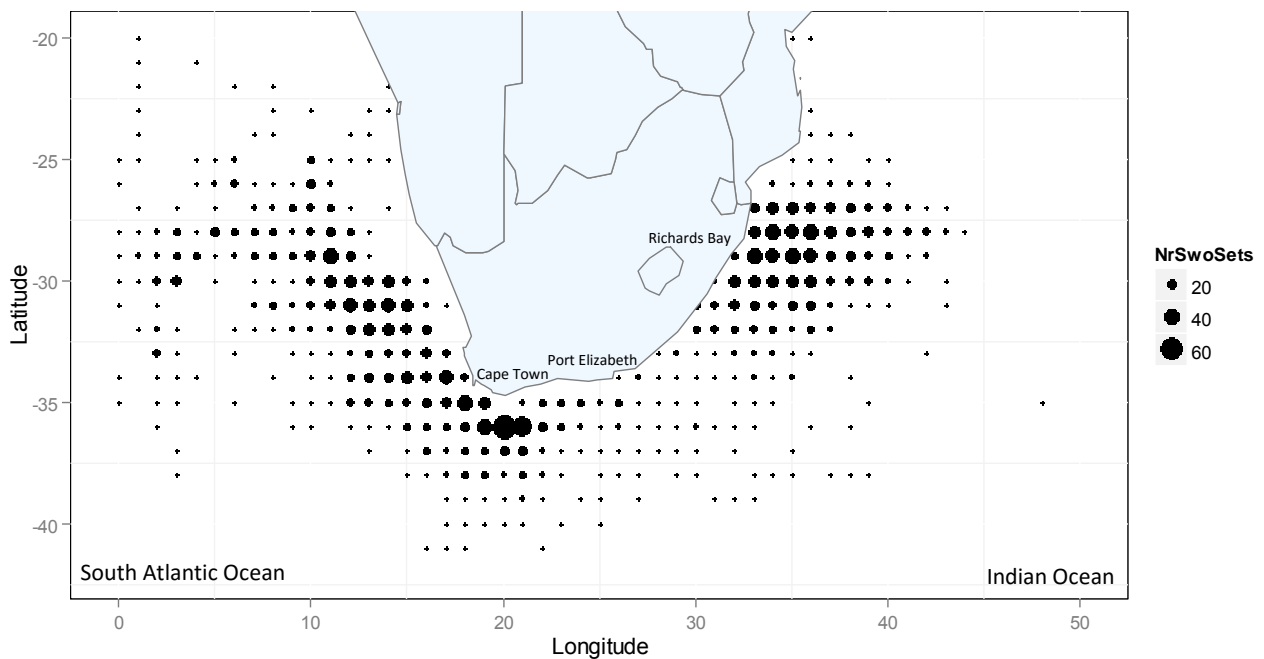


Figure 3. The number of pelagic longline sets that caught swordfish per 1x1° block from 1997 – 2013.

### *Definition of a stock*

A genetic stock is defined as a group of organisms that have the capability to interbreed and maintain the Hardy-Weinberg equilibrium (Booke, 1981). Fisheries scientists however accept the more general ecological/biological stock (synonymous with 'population') of semi-discrete groups of fish with some definable attributes of interest to managers (Begg *et al.*, 1999). This stock definition implies that each unit has homogenous life history characteristics (*e.g.* growth, size at maturity, fecundity *etc.* and a shared mortality rate), each being discrete (no emigration or immigration) and self-recruiting, in a defined area even though the units may be genetically homogenous (Hillborn and Walters, 1992; Begg and Waldman, 1999). Furthermore, a management stock may refer to a biological stock or a multispecies complex that is managed as a single unit (NOAA, 2012).

Random dispersal and directed migrations due to seasonal or reproductive activity can occur, and this can create challenges in determining stock boundaries. Fisheries scientists and managers need to revisit definitions of stock boundaries regularly as the stock abundance and distribution changes or as break-through technologies become available. Fish stocks need to be identified accurately to ensure optimal utilisation and conservation of the natural resources (Begg and Cadrin, 2009).

### *Swordfish stocks*

Swordfish is a globally distributed species that occurs in six recognised stocks, the - Mediterranean, North Atlantic, South Atlantic, Indian, East Pacific and West and South Pacific stocks. Spatial heterogeneity in stock indicators and localised stock depletion has led to an additional management sub-unit of swordfish caught in the South West Indian Ocean region. Since swordfish distribution within each stock extends beyond national and political boundaries, inter-governmental tuna Regional Fisheries Management Organisations (tRFMOs) were made responsible for the conservation of tuna and tuna-like species (including swordfish) through the efforts of member parties. The tRFMOs include the 1) Commission for the Conservation of Southern Bluefin Tuna (CCSBT), 2) Inter-American Tropical Tuna Commission (IATTC), 3) ICCAT, 4) Indian Ocean Tuna Commission (IOTC) and 5) Western and Central Pacific Fisheries Commission (WCPFC). The tRFMOs study and manage the stocks through the collection and statistical analysis of information (from member and participating countries) relative to current conditions and trends of the fishery resources. Fisheries scientists conduct stock assessments to provide managers with the tools to

provide advice for the sustainable management of the stocks. The management advice could include quotas, size limits, closed seasons and areas, and restrictions on fishing effort or gear.

Defining a stock is vital to accurately determine the past and current status of the stock, the rates of recruitment and mortality, the reaction to increased or decreased fishing pressure, the Maximum Sustainable Yield (MSY) and to inform fisheries managers how best to manage the stocks (Cooper, 2006). Different outcomes in stock assessments are expected between a scenario with a single, exploited population and a scenario in which multiple, demographically independent populations are exploited simultaneously (Carvalho and Hauser, 1994). In the latter case, treating two or more independent fishery stocks as a single unit could potentially lead to underestimates of the effects of exploitation on local stocks and an overall diminished yield (Carvalho and Hauser, 1994).

Stock assessments require that the 1) stock structure is clearly defined and understood and 2) that there is an index of abundance. Management of wild populations is improved and stock assessments of marine species are improved when genetic data are used in the development of a fishery stock definition (Begg and Waldman, 1999; Waples *et al.*, 2008). Therefore, genetic analyses of population structure can improve stock assessments by increasing accuracy in identification of the number of populations exploited and the extent and direction of demographic exchange (migration) among exploited populations (Begg and Waldman, 1999; Waples *et al.*, 2008).

Although genetics techniques may be used to establish the ecological stock structure and to quantify the rate of gene flow and interbreeding among ecological stocks, these should not be used as a definitive indication of stock structure (Appleyard *et al.*, 2002; Cheng *et al.*, 2015). Marine ecosystems, however big or small, have environmental boundaries such as temperature or habitat. If a species is able to overcome these boundaries then a degree of stock mixture is inevitable. The extent of stock mixture is exacerbated in oceanic migrants such as tunas and billfish that occur in ocean basins globally. Stock assessments are based on the assumption that the stock is discrete and self-recruiting (Sparre and Venema, 1998). Estimations of important population parameters such as spawning stock biomass, recruitment and sustainable yield would not be accurate if a degree of mixing between stocks has not been accounted for in stock assessments. Population genetics analyses have the potential to detect population structure and population divergence of swordfish around the South African coastline with the use of genetic markers such as mitochondrial DNA that can detect the evolution of the species and microsatellites that are able to detect contemporary changes in the recent past (André *et al.*, 2011). Genetic population structure and divergence results

will inform scientists and managers on the South Atlantic and Indian Ocean swordfish stock dynamics in this area, and its implications for the current management boundaries and strategies for this important commercial species.

South Africa straddles two ocean basins, the Indian and Atlantic Ocean, and two major current systems, the Agulhas and Benguela currents, which have created two main biogeographical regions in the inshore area between the east and the west coasts of the country (Lutjeharms, 2007). Currently the jurisdictions of IOTC and ICCAT are separated by a management boundary at 20°E. Consequently, all tunas and billfish stocks with exception of the southern bluefin tuna, are artificially divided into Atlantic and Indian Ocean stocks along this boundary, regardless of their ecological and biological boundaries. As early as 1991, Mejuto and Hoey (1991), stated that “the possibility of a relatively coastal communication between the Indian Ocean and the South Atlantic, between Subtropical Convergence and the Cape Good Hope zone, favoured by the season variations of the Agulhas Current, should be considered.”

There are a number of biological (temperature preferences, feeding and spawning behaviour) and environmental (food availability, currents, oxygen concentration, temperatures, spawning and feeding grounds) components that affect the movement patterns of large pelagic species, including swordfish. There are also many multidisciplinary approaches used to determine the migratory behaviour of fish, either directly or indirectly. These include studies on tagging (conventional, acoustic and satellite), organic traces (lipids, proteins), chemical traces (PCBs, DDTs), otolith microchemistry, feeding (stomach contents), and spawning (gonad maturity and spawning season) (Appleyard *et al.*, 2002; Cheng *et al.*, 2015).

#### *Abundance index and stock status*

Stock assessments commonly require at least an index of abundance and a catch data series. The index of abundance indicates the relative abundance change of a species over time and is proportional to the true stock size (Cooper, 2006). As it indicates relative abundance, in isolation it can inform management, especially in data poor fisheries. Additional data such as the proportionality estimate (catchability coefficient,  $q$ ) will assess the absolute change in the stock and can deduce the status of the stock (Cooper, 2006; Maunder *et al.*, 2006). An example of an index of abundance would be the kilograms of tuna caught per vessel per day, otherwise referred to as catch-per-unit-effort (CPUE). For the indices of abundance to be comparable over time, the conditions

under which the indices were estimated should remain the same. However, when utilising fishery dependent catch data, the distribution of data varies annually and between vessels in respect of areas fished, vessel size and capabilities, gear used, skills level etc. To remove the effect of factors that bias the index of abundance, the raw CPUE data has to be standardised (Maunder *et al.*, 2006). The most common method to standardise CPUE is in the application of the generalised liner model (GLM) (Hinton and Maunder, 2003). The CPUE is predicted as a linear combination of the explanatory variables. The main objective of the analysis is to estimate a year effect by removing as many other sources of variation as possible. The year effect is used to represent the annual relative levels of abundance and the relative index of abundance to include in the stock assessment (Hinton and Maunder, 2003).

Stock assessments for tunas and billfish are conducted on a regional level for each stock by the tRFMOs every 2-3 years. Each fishing country/flag supplies catch data annually to the various tRFMOs for inclusion in stock assessments. Countries may be required to generate a standardised CPUE using their catch data for a particular species and stock. There are many papers detailing the standardised CPUE for swordfish by participants in tRFMO stock assessments (*e.g.* Chang *et al.*, 2007; Ortiz, 2007; Semba *et al.*, 2008; Kolody, 2011; Nishida *et al.*, 2011; Kai and Yokawa, 2014; Ramos-Cartelle *et al.*, 2014; Santos *et al.*, 2014). The latest swordfish stock assessments conducted by ICCAT and IOTC were in 2013 and 2014, respectively. The stock assessment usually places importance on data from countries with a large fleet that fishes wide areas over a long time scale, and will consider including data from younger or smaller fishing fleets as secondary priority.

MSY is, theoretically, the largest long-term average yield (or catch) that can be taken from a stock under prevailing conditions without impairing on its renewability (Maunder, 2008). The status of a stock can be measured by the ratios of current fishing mortality rate ( $F$ ) and spawner biomass ( $SB$ ) to that of the fishing mortality that will lead to MSY (i.e.  $F_{MSY}$ ) and the spawner biomass that will lead to MSY (i.e.  $SB_{MSY}$ ).

There remains uncertainty around the current stock status of swordfish in the South Atlantic Ocean. The most conservative estimate is that the stock is not undergoing overfishing ( $F_{2011}/F_{MSY} < 1$ ) and is fully exploited ( $B_{2011}/B_{MSY} < 1$ ) (ICCAT, 2015).

Currently swordfish in the Indian Ocean is not subject to overfishing ( $F_{2013}/F_{MSY} < 1$ ) and is not overfished ( $SB_{2013}/SB_{MSY} > 1$ ) (IOTC, 2015). The inclusion of South Africa's data (i.e. indices of

abundance) in these stock assessments comes with reservation. There is uncertainty over the origin of South African caught swordfish and it is unclear as to which stock is represented by SB and mortality indices in this region.

South Africa is yet to have a standardised CPUE included in the stock assessments of ICCAT and IOTC, which this thesis will address. Swordfish life history characteristics, including a relatively late maturity, long life and sexual dimorphism, make the species vulnerable to over exploitation. The declining trend in catches in South Africa since 2005 could be an indication of local over exploitation and stock depletion. In addition, the inclusion of a South African standardised CPUE would provide an interesting perspective of catches from a largely inshore fishery at the southern limit of the swordfish distribution range. Since there is doubt over the legitimacy of a management boundary at 20°E, the tRFMOs are wary of utilising South African data.

### *Thesis structure*

The second chapter of this thesis is a population genetics study of swordfish sampled across the management boundary that currently separates the two stocks of the Indian and South Atlantic Ocean at 20°E. The results will aid determining the interaction between the recognised biological stocks of the Indian Ocean and South Atlantic Ocean at this boundary zone, and may provide an alternative boundary that is biologically relevant. Microsatellite markers (Muths *et al.*, 2013) will be used to study the genetic differentiation of swordfish straddling the two oceans along the South African coastline. The diversity of individuals, their stock origin, population structure, differentiation and the level of gene flow around South Africa will be analysed.

The third chapter will model the relative abundance index of swordfish caught around South Africa by the pelagic longline fleet. The stock structure results of the second chapter may be used to separate the data into two stocks, and the focus of this chapter will be on a suitable GLM model for the data and understanding the changes in abundance since the inception of the fishery.

The fourth chapter will provide a management perspective by a scientist based on the current management regime overseen by the tRFMOs, and how the results of this study may impact the neighbouring management bodies and their current approach to swordfish stock separation.

## References

- Abascal, F. J., Mejuto, J., Quintans, M. and Ramos-Cartelle, A. (2010). Horizontal and vertical movements of swordfish in the Southeast Pacific. *ICES Journal of Marine Science*, 67(3), pp. 466-474.
- Abascal, F. J., Mejuto, J., Quintans, M., García-Cortés and Ramos-Cartelle, A. (2015). Tracking of the broadbill swordfish, *Xiphias gladius*, in the central and eastern North Atlantic. *Fisheries Research*, 162, pp. 20-28.
- André, C., Larsson, L. C., Laikre, L., Bekkevold, D., Brigham, J., Carvalho, G. R., Dahlgren, T. G., Hutchinson, W. F., Mariani, S., Mudde, K., Ruzzante, D. E. and Ryman, N. (2011). Detecting population structure in a high gene-flow species, Atlantic herring (*Clupea harengus*): direct, simultaneous evaluation of neutral vs putatively selected loci. *Heredity*, 106, pp. 270 – 280.
- Appelyard, S. A., Ward, R. D. and Grewe, P. M. (2002). Genetic stock structure of bigeye tuna in the Indian Ocean using mitochondrial DNA and microsatellites. *Journal of Fish Biology*, 60, pp. 767-770.
- Barclay, K. and Koh, S-H. (2005). Neoliberalism in Japan's tuna fisheries? Government intervention and reform in the distant water longline industry. University of Canberra (Australia). Asia Pacific School of Economics and Government. Available at: [https://www.researchgate.net/publication/47552565\\_Neoliberalism\\_in\\_Japan's\\_tuna\\_fisheries\\_Government\\_intervention\\_and\\_reform\\_in\\_the\\_distant\\_water\\_longline\\_industry](https://www.researchgate.net/publication/47552565_Neoliberalism_in_Japan's_tuna_fisheries_Government_intervention_and_reform_in_the_distant_water_longline_industry). [Accessed: 3 March 2016].
- Begg, G. A., Friedland, K. D. and Pearce, J. B. (1999). Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research*, 43, pp. 1-8.
- Begg, G. A. and Waldman, J. R. (1999). An holistic approach to fish stock identification. *Fisheries Research*, 43, pp. 35-44.
- Begg, G. A. and Cadrin, S. X. (2009). Chapter 6. Stock identification. In: T. Jakobsen, M.J. Fogarty, B.A. Megrey and E. Moksness, ed., *Fish reproductive biology: implications for assessment and management*, 1<sup>st</sup> ed. Chichester: Wiley-Blackwell, pp. 230-253.
- Booke, H. E. (1981). The conundrum of the stock concept – are nature and nurture definable in fishery science. *Canadian Journal of Fisheries and Aquatic Sciences*, 38, pp. 1479 – 1480.
- Carey, F. G. (1982). A brain heater in the swordfish. *Science*. 216 (4552), pp. 1327-1329.
- Carey, F. G. (1990). *Further acoustic telemetry observations of swordfish*. In: Planning the Future of Billfishes—Research and Management in the 90s and Beyond. 2. Contributed Papers. Proceedings of the Second International Billfish Symposium, Kailua-Kona, HI, 1–5 August 1988, pp. 103–122.



- Ed. by R. H. Stroud. National Coalition for Marine Conservation, Inc., Savannah, GA, Marine Recreational Fisheries, 13. 321 pp.
- Carvalho, G. R. and Hauser, L. (1994). Molecular-genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries*, 4, pp. 326 – 350.
- Chang, S-K., Lee, H-H. and Liu, H-I. (2007). Standardization of South Atlantic swordfish by-catch rate for Taiwanese longline fleet. *Collective Volume of Scientific Papers ICCAT*, 60, pp. 1974 – 1985.
- Cheng, J., Yanagimoto, T., Song, N. and Gao, T. (2015). Population genetic structure of chub mackerel *Scomber japonicas* in the Northwestern Pacific inferred from microsatellite analysis. *Molecular Biology Report*, 42, pp. 373 – 382.
- Cooper, A. B. (2006). A guide to fisheries stock assessment. From data to recommendations. 1<sup>st</sup> ed. [pdf.] New Hampshire: NH Sea Grant College Program. Available at: [http://sedarweb.org/docs/page/stockassessmentguide\\_NHSeaGrant.pdf](http://sedarweb.org/docs/page/stockassessmentguide_NHSeaGrant.pdf) [Accessed 1 February 2016].
- Dewar, H., Prince, E. D., Musyl, M. K., Brill, R. W., Sepulveda, C., Luo, J., Foley, D., Orbesen, E. S., Domeier, M. L., Nasby-Lucas, N., Snodgrass, D., Laurs, R. M., Hoolihan, J. P., Block, B. A., Mcnaughton, L. M. (2011). Movements and behaviours of swordfish in the Atlantic and Pacific Oceans examined using pop-up satellite archival tags. *Fisheries Oceanography*, 2, pp. 219–241.
- DTI (2014). The Department of Trade and Industry, Research and Statistics. Available at: <http://www.thedti.gov.za/> [Accessed 20 June 2014].
- Evans, K., Kolody, D., Abascal, F., Holdsworth, J., Maru, P. and Sippel, T. (2012). Spatial dynamics of swordfish in the South Pacific Ocean inferred from tagging data. In: WCPFC document WCPFC-SC8-2012/SA-IP-05, p. 36.
- ©FAO (2016). Global Capture Production. Available at: <http://www.fao.org/fishery/statistics/global-capture-production/en> [Accessed 2 February 2016].
- Fisheries and Oceans Canada (2009). Archived - Canadian Atlantic swordfish and other tunas - 2004-2006. Available at: <http://www.dfo-mpo.gc.ca/fm-gp/peches-fisheries/ifmp-gmp/swordfish-espardon/swordfish-2004-espardon-eng.htm#tphp> [Accessed 1 February 2016].
- Govender, A., van der Elst, R. and James, N. (2003). Swordfish. Global lessons. 1<sup>st</sup> ed. [pdf] South Africa: WWF-South Africa. Available at: <http://hdl.handle.net/1834/921> [Accessed 1 February 2016].
- Hillborn, R. and Walters, C. J. (1992). *Quantitative Fisheries Stock Assessment. Choice, Dynamics and Uncertainty*. London: Chapman and Hall, p. 604.
- Hinton, M. G. and Maunder, M. N. (2003). Methods for standardizing CPUE and how to select among them. *Collective Volume of Scientific Papers ICCAT*, 56, pp. 169 – 177.
- Horne, C. (1959). *Big Game Fishing*. 1<sup>st</sup> ed. South Africa: Timmins, pp. 140 – 150.

- ICCAT (2015). *Report of the Standing Committee on Research and Statistics (SCRS)*. [online] Madrid, Spain: ICCAT, p. 167. Available at: <http://www.iccat.es/en/meetings.asp> [Accessed 1 February 2016].
- IOTC. (2013). Examination of the effects of piracy on fleet operations and subsequent catch and effort trends. IOTC–2013–SC16–13, Available at: <http://www.iotc.org/documents/examination-effects-piracy-fleet-operations-and-subsequent-catch-and-effort-trends> [Accessed 3 June 2016].
- IOTC (2015). Nominal catch by species and gear, by vessel flag reporting country database. Available at: <http://www.iotc.org/documents/nominal-catch-species-and-gear-vessel-flag-reporting-country> [Accessed 1 February 2016].
- Jenkins, J. L. (1988). *Duelling with swordfish in Chile*. Americas, 40, p. 8.
- Kai, E. T. and Marsac, F. (2010). Influence of mesoscale eddies on spatial structuring of top predators' communities in the Mozambique Channel. *Progress in Oceanography*, 86, pp. 214 – 223.
- Kai, M. and Yokawa, K. (2014). CPUE standardization of the South Atlantic swordfish caught by Japanese longliners for 1990 – 2012. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1837 – 1842.
- Kolody, D. (2011). Review of CPUE issues for the 2011 Indian Ocean swordfish stock assessment. [online]. 9<sup>th</sup> Working Party on Billfish, Indian Ocean Tuna Commission, Mahé, Seychelles. *IOTC-2011-WPB09-13*. Available at: <http://www.iotc.org/documents/review-cpue-issues-2011-io-swo-stock-assessment> [Accessed 1 February 2016].
- Lutjeharms, J. R. E. (2007). Three decades of research on the greater Agulhas Current. *Ocean Science*, 3(1), pp. 129-147.
- Maunder, M. N. (2008). Maximum Sustainable Yield. *Encyclopedia of Ecology*, 5, pp. 2292-2296.
- Maunder, M. N., Sibert, J. R., Fonteneau, A., Hampton, J., Kleiber, P., and Harley, S. J. (2006). Interpreting catch per unit effort data to assess the status of individual stocks and communities. *ICES Journal of Marine Science*, 63, pp. 1373 - 1385.
- Mejuto, J. and Hoey, J. J. (1991). An approach to a stock hypothesis for the swordfish (*Xiphias gladius*) of the Atlantic Ocean. *Collective Volume of Scientific Papers ICCAT*, 35(2), pp. 482-501.
- Mejuto, J. and De la Serna, J. M. (2000). Standardized catch rates by age and in biomass for the North Atlantic swordfish (*Xiphias gladius*) from the Spanish longline fleet for the period 1983 – 1998 and bias produced by changes in the fishing strategy. *Collective Volume of Scientific Papers ICCAT*, 51(5), pp. 1387 – 1410.
- Mejuto, J., García-Cortés, B., Ramos-Cartelle, A., De la Serna, J. M., González-Gonzalez, I. and Fernández-Costa, J. (2013). Standardized catch rates of shortfin mako (*Isurus oxyrinchus*) caught

- by the Spanish surface longline fishery targeting swordfish in the Atlantic Ocean during the period 1990 – 2010. *Collective Volume of Scientific Papers ICCAT*, 69, pp. 1657 – 1669.
- Mejuto, J. and García-Cortés, B. (2014). Reproductive activity of swordfish, *Xiphias gladius*, in the Atlantic Ocean inferred on the basis of macroscopic indicators. *Revista de Biología Marina y Oceanografía*, 49(3): pp. 427 – 447.
- Miyake, M. P. (2006). Factors affecting recent developments in tuna longline fishing capacity and possible options for management of longline capacity. *FAO Fisheries Proceedings* 8, Rome, 2007.
- Muths, D., Le Couls, S., Evano, H., Grewe, P. and Bourjea, J. (2013). Multi-genetic marker approach and spatio-temporal analysis suggest there is a single panmictic population of swordfish *Xiphias gladius* in the Indian Ocean. *PLoS ONE*, 8: e63558.
- Nakamura, I. (1985). FAO species catalogue. Billfishes of the World. An annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date. *FAO Fisheries Synopsis*, 5(125): p. 65.
- Neilson, J. D., Smith, S., Royer, F., Paul, S. D., Porter, J. M. and Lutcavage, M. (2009). Investigations of horizontal movements of Atlantic swordfish using pop-up satellite archival tags. In: Nielsen, J. L., Arrizabalaga, H., Fragoso, N., Hobday, A., Lutcavage, M. and Sibert, J., ed., *Tagging and Tracking of Marine Animals with Electronic Devices*. Netherlands: Springer, pp. 145 – 159.
- Neilson, J., Arocha, F., Calay, S., Mejuto, J., Ortiz, M., Scott, G., Smith, C., Travassos, P., Tserpes, G. and Andrushchenko, I. (2013). The recovery of Atlantic swordfish: the comparative roles of the Regional Fisheries Management Organization and species biology. *Reviews in Fisheries Science*, 21(2), pp. 59 – 57.
- Neilson, J.D., Loefer, J., Prince, E.D., Royer, F., Calmettes, B., Gaspar, P., Lopez, Andrushchenko, I. (2014). Seasonal distributions and migrations of Northwest Atlantic swordfish: inferences from integration of pop-up satellite archival tagging studies. *PLoS ONE*, 9: e112736.
- Nepgen, C. S. De V. (1970). Exploratory fishing for tuna off the South African west coast. *Investigational report (South Africa. Division of Sea Fisheries)*, 87, p. 26.
- Nishida, T., Kitakado, T. and Wang, S-P. (2011). Estimation of the Abundance Index (AI) of swordfish (*Xiphias gladius*) in the Indian Ocean (IO) based on the fine scale catch and effort data of the Japanese tuna longline fisheries (1980 – 2010). [online]. 9<sup>th</sup> Working Party on Billfish, Indian Ocean Tuna Commission, Mahé, Seychelles. *IOTC-2011-WPB09-14*. Available at: <http://www.iotc.org/sites/default/files/documents/proceedings/2010/wpb/IOTC-2010-WPB-09.pdf>. [Accessed 1 February 2016].
- NOAA (2012). Fish Stock Assessment 101 Series: Part 1—Data Required for Assessing U.S. Fish Stocks. Available at:

[http://www.nmfs.noaa.gov/stories/2012/05/05\\_23\\_12stock\\_assessment\\_101\\_part1.html](http://www.nmfs.noaa.gov/stories/2012/05/05_23_12stock_assessment_101_part1.html)

[Accessed 1 February 2016].

Ortiz, M. (2007). Update of standardized catch rates by sex and age for swordfish (*Xiphias gladius*) from the U.S. longline fleet 1981 – 2005. *Collective Volume of Scientific Papers ICCAT*, 60, pp. 1994 – 2017.

Penney, A. J. and Griffiths, M. H. (1999). A first description of the developing South African pelagic longline fishery. *Collective Volume of Scientific Papers ICCAT*, 49, pp. 162 – 173.

Poisson, F., Marjolet, C. and Fauvel, C. (2001). Sexual maturity, spawning season and estimation of batch fecundity of swordfish (*Xiphias gladius*) caught by the Reunion-based pelagic longline fishery (SWOI). 2<sup>nd</sup> Working Party on Billfish, Indian Ocean Tuna Commission, St Gilles, Reunion. WPB01-05. Available at:

<http://www.iotc.org/sites/default/files/documents/proceedings/2001/wpb/IOTC-2001-WPB-05.pdf>

[Accessed 1 February 2016].

Poisson, F. and Fauvel, C. (2009). Reproductive dynamics of swordfish (*Xiphias gladius*) in the southwestern Indian Ocean (Reunion Island). Part 1: oocyte development, sexual maturity and spawning. *Aquatic Living Resources*, 22, pp. 45 – 58.

Pons, M., Forselledo, R. and Domingo, A. (2014). Standardized CPUE of swordfish (*Xiphias gladius*) caught by Uruguayan longliners in the Southwestern Atlantic Ocean (1982 – 2012). *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1758 – 1776.

Ramos-Cartelle, A., García-Cortés, B. and Mejuto, J. (2014). Standardized catch rates in biomass for the South Atlantic stock of swordfish (*Xiphias gladius*) from the Spanish longline fleet for the period 1989 – 2011. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1804 – 1811.

Reed, C. and Cannon, W. J. (2009). *Marine Science: Decade by Decade*. Infobase Publishing, p. 298.

Richardson, D. E., Cowen, R. K., Prince, E. D., Sponaugle, S. (2009). Importance of the Straits of Florida spawning ground to Atlantic sailfish (*Istiophorus platypterus*) and blue marlin (*Makaira nigricans*). *Fisheries Oceanography*, 18: pp. 402–418.

Rooker, J. R., Simms, J. R., Wells, R. J. D. , Holt, S. A., Holt, G. J., Graves, J. E., Furey, N. B. (2012). Distribution and Habitat Associations of Billfish and Swordfish Larvae across Mesoscale Features in the Gulf of Mexico. *PLoS ONE*, 7(4): e34180.

Santos, M. N., Coelho, R. and Lino, P. G. (2014). Swordfish catches by the Portuguese pelagic longline fleet between 1998 – 2013 in the Southwest Indian Ocean: effort, standardized CPUE and catch-at-size. 12<sup>th</sup> Working Party on Billfish, Indian Ocean Tuna Commission, Yokohama, Kanagawa, Japan. *IOTC–2014–WPB12–19*. Available at:

- [http://www.iotc.org/sites/default/files/documents/2014/10/IOTC-2014-WPB12-19 - Portuguese SW-IO SWO LL CPUE.pdf](http://www.iotc.org/sites/default/files/documents/2014/10/IOTC-2014-WPB12-19_-_Portuguese_SW-IO_SWO_LL_CPUE.pdf) [Accessed 1 February 2016].
- Saxton, C. (2012). *Oceans Alive: Swordfish*. Bellwether Media, p. 32.
- Sedberry, G. R. and Loefer, J. K. (2001). Satellite telemetry tracking of swordfish, *Xiphias gladius*, off the eastern United States. *Marine Biology*, 139, pp. 355 – 360.
- Semba, Y., Nishida, T. and Wang, S-P. (2008). Preliminary results of standardization of swordfish CPUE of Taiwanese and Japanese tuna longline fisheries in the Indian Ocean (1980- 2006 and 1990-2006). 6<sup>th</sup> Working Party on Billfish, Indian Ocean Tuna Commission, Seychelles. *IOTC–2008–WPB03*. Available at: <http://www.iotc.org/sites/default/files/documents/proceedings/2008/wpb/IOTC-2008-WPB-03.pdf> [Accessed 1 February 2016].
- Smal, J. (2014). *BROADBILL SWORDFISH — Prized gladiator of the deep — Part 2*. Ski-Boat: The South African Deep Sea Angler, 30(1).
- Sparre, P. and Venema, S. C. (1998). Introduction to tropical fish stock assessment. Part 1. Manual. *FAO Fisheries Technical Paper N° 306.1*. 2<sup>nd</sup> ed. Rome: FAO, pp. 407.
- Takashi, M., Okamura, H., Yokawa, K. and Okazaki, M. (2003). Swimming behaviour and migration of a swordfish recorded by an archival tag. *Marine and Freshwater Research*, 54, 527 – 534.
- Taylor, R. G. and Murphy, M. D. (1992). Reproductive biology of the swordfish *Xiphias gladius* in the Straits of Florida and adjacent waters. *Fishery Bulletin*, 90, pp. 809 – 816.
- Tibbo, S. N., Day, L. R. and Doucet, W. F. (1961). The swordfish (*Xiphias gladius* L.), its life history and economic importance in the northwest Atlantic. *Journal of the Fisheries Research Board of Canada*, 130, pp. 20 – 21.
- Waples, R. S., Punt, A. E. and Cope, J. M. (2008). Integrating genetic data into management of marine resources: how can we do it better? *Fish and Fisheries*, 9, pp. 423 – 449.
- Ward, P. (2000). Swordfish fisheries and management today. *Pelagic Fisheries Research Program*, 5, 3pp.
- Ward, P. and Elscot, S. (2000). Broadbill swordfish: Status of world fisheries. Bureau of Rural Sciences, Canberra.
- Welsh, J. G. (1968). A new approach to research on tuna in South African waters. *Fisheries Bulletin of South Africa*, 5: pp. 32 - 34.
- West, W. M., Kerwath, S. E., Da Silva, C., Wilke, C. G. and Marsac, F. (2012). Horizontal and vertical movements of swordfish tagged with pop up-satellite transmitters in the South-West Indian Ocean, off South Africa. 10<sup>th</sup> IOTC Working Party on Billfish. *IOTC-2012-WPB10-16*. [Accessed 15 February 2016].

Young, J., Lansdell, M., Riddoch, S. and Reville, A. (2006). Feeding ecology of broadbill swordfish, *Xiphias gladius*, off eastern Australia in relation to physical and environmental variables. *Bulletin of Marine Science*, 79, pp. 793 – 809.

## CHAPTER 2

# Genetic differentiation and admixture of swordfish (*Xiphias gladius*) around South Africa

## Abstract

The South Atlantic and Indian Ocean stocks of swordfish (*Xiphias gladius*) are currently separated by a management boundary at 20°E that is recognised by the two relevant tuna Regional Fisheries Management Organisations (tRFMOs), the Indian Ocean Tuna Commission (IOTC) and International Commission for the Conservation of Atlantic Tuna (ICCAT). This boundary divides the fisheries data of the fleets that operate across this boundary. A majority of the previous studies on the population structure of swordfish between the Indian and Atlantic oceans have been based on broad geographical scales. Recent genetics studies have confirmed genetic differentiation between the two stocks but there is no agreement on the direction of gene flow and where the genetically relevant boundary is situated. Eleven microsatellite loci were used to study the regional scale population structure of swordfish caught relatively close inshore with muscle material of 267 swordfish sampled in 2005 around the entire range of South Africa's coastline. The muscle samples had desiccated and four deoxyribonucleic acid (DNA) extraction methods were tested for success and feasibility, of which the DNeasy® Blood and Tissue Kit was chosen. Despite the poor quality of the degraded DNA samples, and subsequent loss of a large number of samples, the results of this study provided evidence for admixture of swordfish around the boundary of the Indian and Atlantic oceans. A posterior predictive map of admixture proportions was used to identify a potential admixture zone between 14°E and 27°E. There is evidence of gene flow and migration in this area in both directions, though the evidence for weak differentiation suggests that the Indian Ocean and Atlantic Ocean contain separate stocks and that swordfish stocks coexist around South Africa but return to their ocean of origin to reproduce. The observed weak structure is in a species that has ample opportunity for admixture and genetic homogenisation due to passive drift of larvae and active dispersal of adults that have wide environmental parameter limits that extend across this area. The results from this study are sufficient to motivate for the two tRFMOs, ICCAT and IOTC, to reconsider the boundaries that separate the two stocks and formulate novel ways to divide the catch in the transitional region (more in Chapter 4).

## Literature review

### *Introduction to population genetics*

Population genetics is the study of the state of a natural population and its evolution through the changes in frequencies of alleles and genotypes (Gillespie, 1998). Since the evolution of a population cannot be observed directly, population genetics is based on the application of theories. Mathematical models of evolution are used to test whether the state and structure of populations' matches the behaviour outlined in the model and to study the evolutionary forces acting on populations (Gillespie, 1998). One such theory, that is the basis of population genetics studies is the Hardy-Weinberg law. The Hardy-Weinberg (HW) law states that phenotypic and allelic frequencies remain constant from generation to generation in sexually reproducing populations if the population meets the conditions of large effective population size, no mutation, no natural selection, migration (*i.e.* no gene flow), no genetic drift, random mating and random reproductive success. A population is said to be in Hardy Weinberg Equilibrium (HWE) when these conditions are met. Natural populations, however, rarely experience HWE. Natural selection ensures that mating and reproductive success is not random, large populations are rarely found in isolation, and all populations experience some level of mutation. The law is tested by measuring the differences between actual and expected allelic frequencies or phenotypes (Edwards, 2008).

The genetic composition of natural populations may change over time. Natural selection, mutation, migration and genetic drift are the mechanisms of evolution. Natural selection selects for advantageous alleles in the population and mutations produce new alleles. Genetic drift results in random fluctuations in allele transfer to offspring in adverse environmental conditions (bottleneck effect) or geographical separation (founder effect) of small populations (Provine, 2004).

Genetic variation between individuals and biological/ecological stocks are constantly under the influence of these factors. Patterns of genetic diversity or variation among populations can provide clues to the populations' life histories and degree of evolutionary isolation (Hellberg, 2009). The rate of gene flow between populations depends both on the dispersal ability of the organism and on the probability that migrants can successfully mate with residents. The degree to which this reproductive isolation can impede genetic exchange between populations of the same species can be assessed by studying both migration and gene flow (Lessios and Weinberg, 1993). Genetic variation between biological stocks are positively associated with time since divergence of stocks (mediated by generation time, with shorter generation time accelerating genetic differentiation), and their degree of isolation (*i.e.* reproductive exchange between stocks eroding genetic differences) (Adkinson, 1996). Thus, recent divergence or substantial secondary reproductive contact results in



no apparent differences in gene frequencies between groups, even when it exists (Begg and Waldman, 1999). Genetic divergence in migratory species may exist if, for example, individuals are philopatric and consistently return to the same breeding grounds (Muths *et al.*, 2013).

#### *Markers used in population genetics*

There are three commonly-used genetic markers – proteins, mitochondrial DNA and nuclear DNA - used in population genetic and phylogenetic studies (Ward, 2000; Okumus and Ciftci, 2003). Proteins detect genetic variation at the level of enzymes. The allelic variants are protein variants called allozymes that differ slightly in electrical charge. Proteins have wide applications, including measuring outcrossing, population structure and population divergence, though they exhibit low levels of polymorphism.

Mitochondrial DNA (mtDNA) is the <1% of DNA found outside of the nucleus and in the mitochondria organelle. This molecule is maternally inherited, the entire genome is transcribed as a unit, it is not subject to recombination and is mainly selectively neutral (Billington, 2003). The characteristics of mtDNA make this molecule ideal for evolutionary studies (Castro *et al.*, 1998). In the nuclear genome, there are segments of DNA that are repeated tens to hundreds and thousands of times (O'Reilly and Wright, 1995). Two classes of these polymorphic repeating segments can be distinguished, a) minisatellite DNA, which refers to genetic loci with repeats of 9-65 base pairs (bp), and b) microsatellite DNA, in which the repeat unit is generally only 2 - 6 bp.

Microsatellites are a more powerful tool than proteins and mitochondrial DNA in defining the geographical and spatial scales for population differentiation as well as in identifying the origins of individuals in mixed stocks of migratory fish (Selkoe and Toonen, 2006). Microsatellites had been the popular marker of choice until the mid-2000s when single nucleotide polymorphism (SNP) markers appeared on the scene (Herráeza *et al.*, 2005). In the last 10 years SNPs have grown in popularity in various fields, including population genetics. These markers represent single base-pair differences in the DNA sequence of individual members of a species. They are predominantly bi-allelic and their small size increases genotyping success and reliability, which is an advantage particularly with degraded DNA (Fabbri *et al.*, 2012).

Microsatellites (also referred to as simple sequence repeats (SSRs), short tandem repeats (STRs) or simple sequence repeat polymorphisms (SSRPs)) are codominant (heterozygotes and homozygotes

can be detected, providing maximum genetic information), highly polymorphic (evolving faster than mtDNA), bi-parentally inherited and susceptible to mutations. The microsatellite repeating sequences repeat 10-100 times. The repeating sequence, found at a particular locus, forms a marker. To be used as markers, their location in the genome of interest must be identified first. Microsatellite markers are polymerase chain reaction (PCR) based, meaning only tiny amounts of tissue are required and has the potential to even be utilised on degraded DNA. Polymorphisms in the repeat sequence can be detected by performing a PCR with primers designed from the DNA flanking region. The result of microsatellite loci sequencing is genotypes of varying sizes (each constituting a different allele) of the repeat sequence when comparing individuals (Jarne and Lagoda, 1996; Ellegren, 2004). Microsatellites can be used to estimate effective population size, detect migrations and infer recent bottlenecks (Morin *et al.* 2012). Microsatellite markers are also useful at a range of scales, from low-level population subdivision to phylogeography (Morin *et al.*, 2012).

#### *Genetic population structure of large pelagic species*

The apparent genetic homogeneity of many marine species is commonly thought to be due to two factors that minimize accumulation of genetic differences among populations: large effective population sizes that limit genetic drift (De Woody and Avise, 2000) and life history characteristics that favour dispersal (*e.g.* planktonic eggs and larvae, juvenile and adult vagility) in continuous dynamic oceanic environments (Ward *et al.*, 1994; Shaklee and Bentzen, 1998; Waples, 1998; Cheng *et al.*, 2015). Nevertheless, considerable evidence has accumulated to show that many marine fish species often exhibit weak but significant population structuring at neutral loci, such as microsatellites (O'Reilly *et al.*, 2004).

A variety of different genetic approaches have been used to investigate biological population structure in migratory pelagic species, refining the methods and analyses over time. There is an abundance of previous studies on large pelagic species, including yellowfin tuna (*Thunnus albacares*), bigeye tuna (*Thunnus obesus*), albacore tuna (*Thunnus alalunga*), Atlantic bluefin tuna (*Thunnus thynnus*), skipjack tuna (*Katsuwonus pelamis*), marlins and spearfish and others investigating genetic differentiation between and within the Indian, Pacific, and Atlantic oceans and the Mediterranean Sea (Appendix A). Of the 47 studies listed, 25 utilised microsatellite loci exclusively or in conjunction with other markers to study population structure. Twelve of the studies investigated the pattern of genetic differentiation of large pelagic species between the Atlantic and Indian oceans.

The study of Ward *et al.* (1997) found distinct stocks of yellowfin tuna between the Indian and Atlantic oceans. However, Ely *et al.* (2005) found a lack of inter-oceanic differentiation in skipjack tuna and yellowfin tuna between the Atlantic and Indo-Pacific regions that the study attributed to extremely large population sizes causing slower genetic drift than that observed for other tunas and that the time since population expansion had not been sufficient to allow for the populations to become differentiated. Alvarado Bremer *et al.* (1998) and Chiang *et al.* (2008) discovered two distinct clades of bigeye tuna between the Indian Ocean and Atlantic Ocean, with the former study acknowledging the contact of the populations around the Cape of Good Hope. Chow *et al.* (2000) and Durand *et al.* (2005) mirrored these results stating that gene flow and fish migration between the Atlantic and Indian oceans were severely restricted, and that fishes from these distinct stocks were intermingling around South Africa. In contrast, Gonzalez *et al.* (2008) reported a single worldwide panmictic stock for bigeye tuna and that despite the strong Agulhas Current, migration rates of relatively low numbers of migrants per generation seemed to be higher from the Atlantic Ocean into the Indo-Pacific Ocean around the Cape of Good Hope. Albaina *et al.* (2013) and Laconcha *et al.* (2015) reported genetically distinct albacore populations of the Indian and Atlantic oceans, in contrast to Montes *et al.* (2012) that found the two populations to be genetically indistinguishable. Blue marlin and sailfish between the Atlantic and Indo-Pacific Ocean were found to be distinct populations according to Graves and McDowell (2003).

The contrasting results of the same species among studies that have differed in the markers used and the sampling regime, stress the importance of considering and incorporating multidisciplinary studies (such as life history characteristics, movement and biology) when determining the biological population structure of a species. Each species is unique in its population size, movement behaviour, mutation rate and response to environmental conditions, and for these reasons the population structure of similar species in a region cannot be generalised.

#### *Previous swordfish genetic stock structure studies*

The current global stock structure of swordfish has been partly elucidated thus far (Kasapidis *et al.*, 2008). Swordfish stocks are divided on an ocean-basis level; two stocks in the Atlantic (North and South), a distinct Mediterranean stock, an Indian Ocean stock (with stock assessment and management advice provided separately for the Southwest Indian Ocean region population) and four Pacific Ocean stocks (Northwest, Northeast, Southwest and Southeast) (Hinton and Alvarado

Bremer, 2007). Uncertainty on the level of mixture between stocks and the boundaries between stocks remains to be resolved (ICCAT, 2013; IOTC, 2013).

There have been a variety of previous studies on the genetic stock structure of swordfish from the 1990s to the present. Allozyme, nucleic DNA (including microsatellites), mtDNA or a combination of markers have been used to determine the population differentiation of swordfish populations between (and within) the Atlantic, Pacific and Indian oceans and the Mediterranean Sea (Appendix A).

Inter-oceanic studies on a global scale have mostly been in agreement on the differentiation of swordfish populations between the North Atlantic, South Atlantic and Pacific oceans and the Mediterranean Sea (Alvarado Bremer *et al.*, 1996; Rosel and Block, 1996; Alvarado Bremer *et al.*, 1998; Chow *et al.*, 1997; Greig *et al.*, 1999; Kotoulas *et al.*, 2003; Smith and Alvarado Bremer, 2010). Studies among neighbouring areas such as North Atlantic-South Atlantic (Chow *et al.*, 2002; Alvarado Bremer *et al.*, 2005; Kasapidis *et al.*, 2006; Chow *et al.*, 2007; Garcia *et al.*, 2011), western Australia-eastern Australia (Ward *et al.*, 2001), Atlantic-Mediterranean (Viñas *et al.*, 2007; Patarnello *et al.*, 2007), Indian-Pacific (Lu *et al.*, 2006) and Mediterranean-North Atlantic-South Atlantic (Smith and Alvarado Bremer, 2010; Smith *et al.*, 2015) have investigated genetic differentiation and the levels of gene flow, particularly in reference to the recognised management boundaries, and proposed reconsiderations. Ward *et al.* (2001) reported differing results of mitochondrial DNA and microsatellite loci used to study the genetic differentiation among eastern- and western Australia and Reunion Island. No statistically significant differences among the three areas were detected for mitochondrial DNA. However, minor but significant differentiation recorded by the AMOVA / $F_{ST}$  method (based on five microsatellite loci) was due to minor differentiation of the Western Australian population from the two other collections, at microsatellite locus Xg-144.

Kasapidis *et al.* (2006) detailed that the South Atlantic individuals were significantly different from the North Atlantic counterparts. In addition, Chow *et al.* (2007) observed that gene flow and individual migration between the North and mid-South Atlantic populations was considerably restricted. According to Alvarado Bremer *et al.* (2005) the mitochondrial DNA distinctiveness of the North West Atlantic and South Atlantic swordfish populations is indicative of philopatric behaviour in swordfish towards breeding and feeding areas. The Atlantic-Mediterranean transition zone and the Strait of Gibraltar as a phylogeographical break have been studied extensively. Viñas *et al.* (2007) reported that the boundary of the Mediterranean stock exceeds the Strait of Gibraltar and a degree

of stock mixture with the Northeast Atlantic can be found. Patarnello *et al.* (2007) reviewed the many previous studies in the Atlantic-Mediterranean transition zone and the results pointed towards a combined signature of vicariance, palaeoclimate fluctuation and life-history traits on the Atlantic-Mediterranean phylogeographical patterns.

The intra-oceanic studies, particularly those in the Pacific Ocean (Hinton and Alvarado Bremer, 2007) and Mediterranean Sea, have produced varying conflicting results; one panmictic Pacific Ocean stock (Grivalja-Chon *et al.*, 1994) and Mediterranean stock (Pla *et al.*, 1998; Viñas *et al.*, 1998) versus low genetic differentiation (Kasapidis *et al.*, 2008) and heterogeneous stock structure (Reeb *et al.*, 2000; Alvarado Bremer *et al.*, 2006; Viñas *et al.*, 2010). Jean *et al.* (2006) and Muths *et al.* (2009) reported one panmictic population of swordfish in the South West Indian Ocean (SWIO), though some weak differentiation seemed to exist within this region.

### *Focus of this research*

There are essentially three principle models of population structure, which can result in differentiation of genetic patterns within and between geographic localities (May and Krueger, 1990; Baverstock and Moritz, 1996). Firstly the “panmictic model”: the entire population may consist of single panmictic unit (free exchange); second, “stepping-stone” or “island model”: the samples may consist of a series of small subpopulations each largely isolated from other subpopulations (no interchange between subpopulations); and third, the “isolation-by-distance” model: a continuous population, in which organisms only exchange genes with geographically close areas (local interchange only) (Ciftci and Okumus, 2002). A caveat to the third model is a continuous population that experiences natal homing whereby individuals will return to the same spawning site in successive years.

The stock structure of fish have been studied using different classes of molecular markers, marker preferences changing depending on the studies’ aims and as the techniques and understanding of the markers evolve, in the process improving our understanding of stock structure. A drawback of genetic stock structure studies for fisheries managers is that genetic homogeneity between two areas does not necessarily mean biological stock homogeneity (Ward, 2000). Conversely, though there may be evidence of genetic differentiation between populations, genetic studies are not conclusive and the importance of multidisciplinary studies (physiological, ecological, and hydrographical information such as tagging, morphology, otolith chemistry, life histories and catch

rates) are suggested to inform and create holistic management of the stocks (Appleyard *et al.*, 2002; Cheng *et al.*, 2015).

The potential for egg and larval dispersion over large distances and adult migratory behaviour in marine environments where physical barriers are often absent lowers genetic differentiation (Ward, 2000; Knutsen *et al.*, 2003). For these reasons, distinct populations are more difficult to detect in the marine environment and for many marine organisms it is unclear to what degree distinct populations exist at all, or whether they are organized into larger panmictic units (McQuinn, 1997). Ward *et al.* (1994) reviewed the total gene diversity ( $F_{ST}$ ) of 113 marine, anadromous and freshwater fish species and found that marine fish species had a mean  $F_{ST}$  of 0.062, considerably lower than anadromous and freshwater fish species (Waples, 1998). This is probably because there are potentially fewer barriers to migration that lead to higher rates of gene flow (Carvalho and Hauser, 1994).

## Introduction

The non-genetic evidence of swordfish structure has been studied using data such as catch rates, catch-at-age and catch-at-length data within ocean basins such as the Atlantic (Neilson *et al.*, 2007) and Pacific (Hinton and Alvarado Bremer, 2007), using tag recapture studies (García-Cortés *et al.*, 2003; Holdsworth *et al.*, 2007), growth parameters (Tserpes and Tsimenides, 1995; Arocha and Lee, 1996) and spawning areas (Amorim and Arfelli, 1980; Arocha and Lee, 1996). Biological data has also been useful in informing management of the species' response to fisheries related measures (Neilson *et al.*, 2013). Challenges conducting multidisciplinary research exist; sampling has to be extensive and biological sampling and tagging for cosmopolitan species such as swordfish over large spatial and temporal scales is costly.

Various studies, mostly on broader geographical scales, have included investigations into the population structure of swordfish between the Indian and Atlantic oceans, which is the focus of the current study as well. The early study by Chow *et al.* (1997) reported that Indian Ocean samples were not significantly different from the samples of South Atlantic and Pacific oceans. Subsequent studies detailed genetic differentiation between the Indian and South Atlantic oceans (Alvarado Bremer *et al.*, 1999; Chow and Takeyama, 2000; Kotoulas *et al.*, 2006; Alvarado Bremer *et al.*, 2007). According to Chow and Takeyama (2000), the penetration by Indo-Pacific swordfish into the Atlantic seemed negligible and the spawning grounds of the Atlantic and Indo-Pacific were separated.

Similarly, Alvarado Bremer *et al.* (2007) concluded that there was likely historical gene flow from the Indo-Pacific to the Atlantic, though currently the gene flow was restricted.

The study by Muths *et al.* (2013) is the most recent study conducted on a relatively regional geographical scale comparing 177 samples collected at the Cape of Good Hope and Namibia (*i.e.* Atlantic) with 812 samples from the SWIO region using mitochondrial DNA (ND2) and 19 microsatellite loci. The SWIO samples with the closest proximity to the Atlantic samples were from Mozambique (n=115) and South Madagascar (n=228). The overall value of F-statistics for ND2 sequences confirmed that the Atlantic and Indian Ocean swordfish represented two distinct genetic stocks. Indo-Pacific differentiation was also significant but lower than that observed between the Atlantic and Indian Ocean. The co-occurrence of two genetic clades, previously only one in the Atlantic, could be explained by unidirectional gene flow from the Indo-Pacific into the South Atlantic. The fact that a second clade was now observed in the Indian Ocean (at the low frequency of 2% but in all the Indian Ocean areas) seems to suggest that a flux of Atlantic swordfish into the Indian Ocean could also occur. The results of their study had management implications for the tRFMOs to consider. The current study is an extension of the Muths *et al.* (2013) study. Muths *et al.* (2013) was limited by broad sampling that could not address the validity/accuracy of the 20° boundary or determine the spatial extent of intermixing. This study will utilise the same microsatellite markers and will extend on findings through a large sampling effort on a finer sampling scale along the entire South African coastline.

For swordfish 63 microsatellite markers exist (Benson *et al.*, 2009), 51 of which have been characterised in publications (Reeb *et al.*, 2003; Kasapidis *et al.*, 2009; Bradman *et al.*, 2011). Since swordfish microsatellite markers have been established and used in previous studies and as microsatellite markers are widely used and considered suitable for studies on population differentiation and regional population structure in swordfish (Reeb and Block, 1997), this type of marker was deemed suitable for this study.

Swordfish have a latitudinal range of between 25°N to 45°S and 60°N to 45°-50°S in the Indian and eastern Atlantic oceans, respectively (Nakamura, 1985). If a geographical scale that is well within the dispersal ability of swordfish was not chosen, any differentiation at such a restricted geographical scale of 25° – 40°S and 10° – 35°E of this study may be interpreted as an innate tendency for population sub-structuring and not just a consequence of external forces (Knutsen *et al.*, 2003). There are no physical barriers or geographical distance separating the stocks of the Indian and

Atlantic oceans in this region. This chapter will attempt to address whether genetic stock differentiation exists for swordfish caught off the South African coastline straddling the management border (20°E) of the Indian and Atlantic oceans with the use of microsatellite markers. Following on from the study by Muths *et al.* (2013), the aim of this study is, therefore, to determine the genetic boundary (if any) between the two management stocks and to quantify the level of gene flow at this boundary.

## Materials and Methods

### *Sample collection*

Observers onboard pelagic longline vessels collected 602 swordfish muscle samples throughout 2005. Samples were collected around South Africa's coastline from -24° to -38° S and 10° to 35°E, sampling the Indian and Atlantic oceans; and were stored in 70% ethanol. The catch location was recorded and the sex identified in all samples (Figure 1).

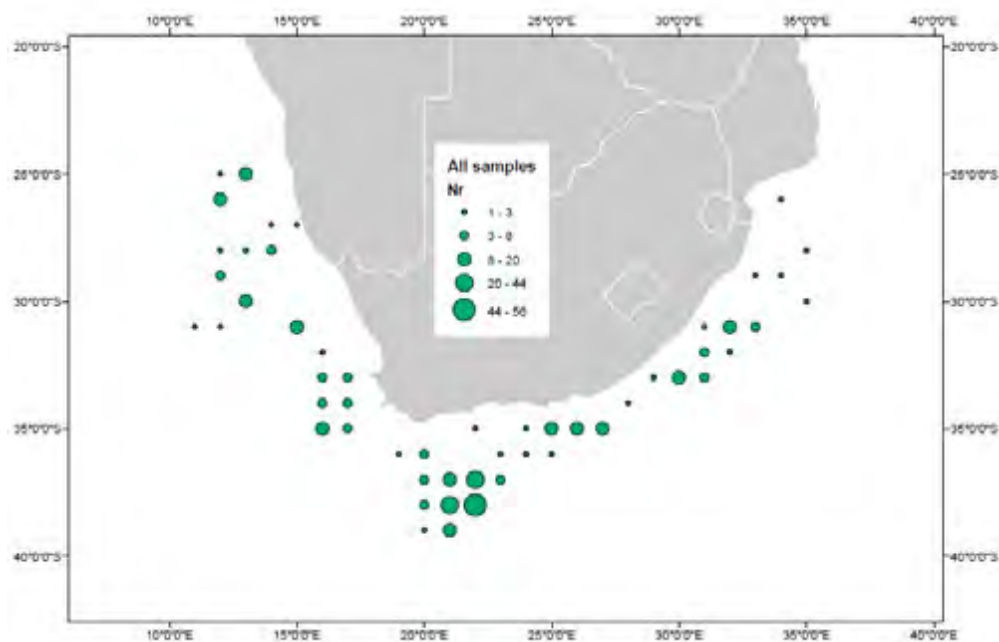


Figure 1. Catch location of all samples collected by onboard observers in 2005.

The management boundary at 20°E that separates the Indian Ocean and Atlantic Ocean stocks was initially used to assign samples to localities, referred to as Scenario 1 (Figure 2, i). Scenario 1 was used for marker performance and genetic diversity analyses. Samples caught west and east of the 20°E boundary are classified West (<20°E) and East (>20°E), respectively.

### *Putative population scenarios*



The individuals were subsequently divided into 3 additional population scenarios based on the longitude geographic parameter to vary the potential stock boundaries and compare the results of population differentiation and assignment tests (Figure 2, ii-iv).

Scenario 2. West (<17°E), South (17°E -30°E), East (>30°E)

Scenario 3. West (<17°E), East (>17°E)

Scenario 4. West (<30°E), East (>30°E)

The boundary lines were chosen to reflect the westerly extent of the Agulhas Current Retroflexion at 17°E and the westerly extent of the Mozambique Channel at 30°E. The number of samples per scenario is included in Table 2. Sample filtering will be applied later on in the study based on the quality of the sample for genetics analyses. The final number of individuals is indicated in the Results.

Table 2. Number of individuals per scenario included in analyses.

Scenario		Scenario code	Number of individuals
1	West (<20°E)	W20	73
	East (>20°E)	E20	194
2	West (<17°E)	W17	62
	South (17-30°E)	South	180
	East (>30°E)	E30	25
3	West (<17°E)	W17	62
	East (>17°E)	E17	205
4	West (<30°E)	W30	242
	East (>30°E)	E30	25

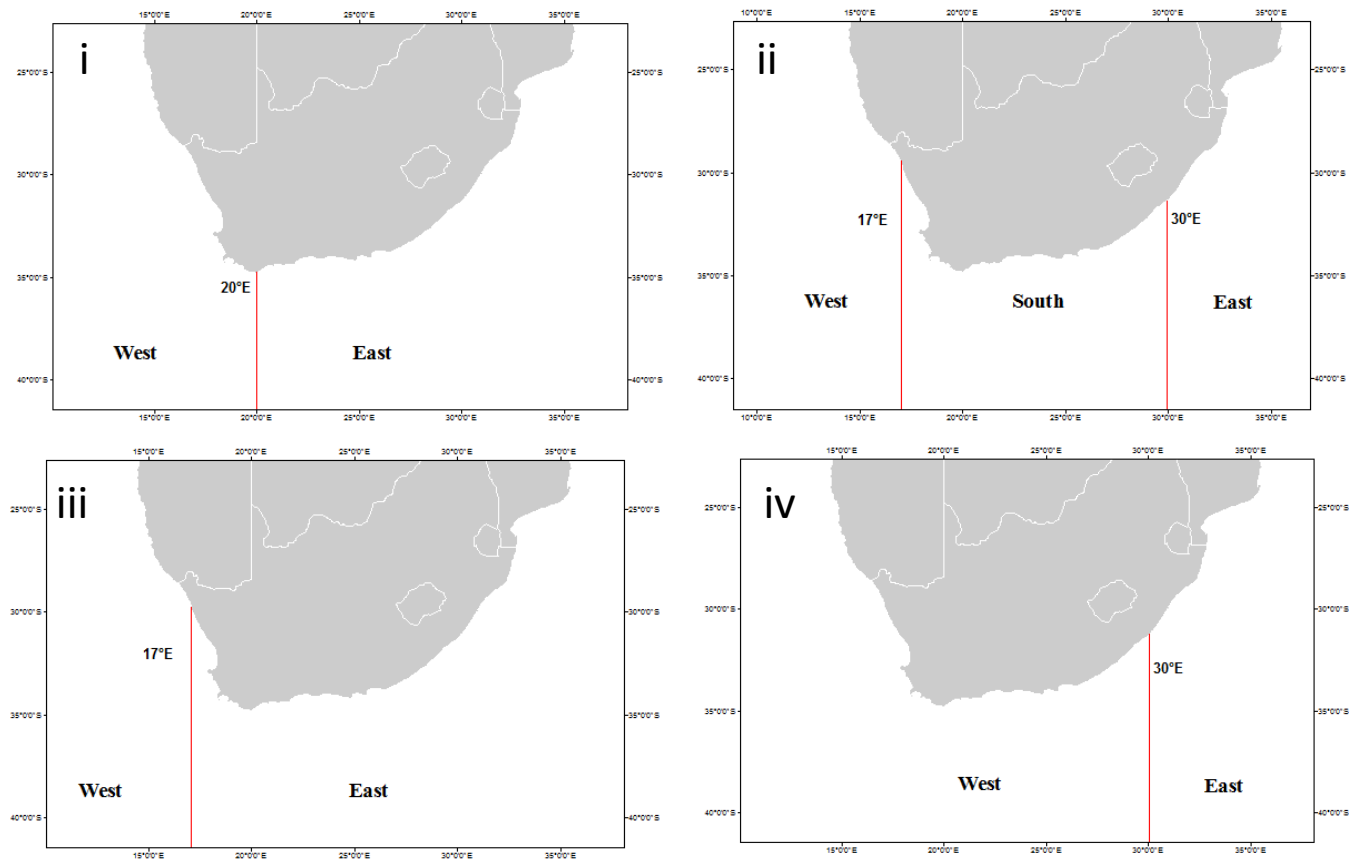


Figure 2. Four population scenarios, i) Scenario 1: West ( $<20^{\circ}\text{E}$ ), East ( $>20^{\circ}\text{E}$ ) according to current management boundaries, ii) Scenario 2: West ( $<17^{\circ}\text{E}$ ), South ( $17^{\circ}\text{E} - 30^{\circ}\text{E}$ ), East ( $>30^{\circ}\text{E}$ ), iii) Scenario 3: West ( $<17^{\circ}\text{E}$ ), East ( $>17^{\circ}\text{E}$ ), and iv) Scenario 4: West ( $<30^{\circ}\text{E}$ ), East ( $>30^{\circ}\text{E}$ ).

### *Comparison of DNA extraction methods*

Due to the age of the samples, the ethanol in the sample vials evaporated over time and all samples were desiccated. Three commercial extraction kits, SureFood® PREP, Qiagen DNeasy® mericon Food Kit, Qiagen DNeasy® Blood & Tissue Kit, and a modified Cetyltrimethyl ammonium bromide (CTAB) extraction protocol (Saghai-Marooof *et al.*, 1984) were used to test the efficiency of extracting DNA from desiccated tissue samples. Efficiency was based on DNA quantity (ng/ $\mu\text{l}$ ) and DNA purity (260/280 nm ratio and 260/230 nm ratio of absorbance). Ratios of 1.8 and 2.0-2.2, respectively, are generally accepted as values for “pure” DNA (T042 Technical Bulletin). The DNA quantity and purity were measured using the Nanodrop® ND-1000 spectrophotometer.

The four extraction methods were tested on 10 samples (A to J). Since there was limited time in which to process 602 samples, a kit rather than the CTAB method was the preferred option for extractions. Three of the 10 samples, of varying DNA yield, that were extracted with the DNeasy® Blood and Tissue Kit were tested for PCR success and to determine the minimum DNA yield

threshold for PCR success. Markers A3 and A8, two markers of varying size range (bp), were chosen to test for PCR success of desiccated samples.

#### *DNA extraction*

After testing and found to be the most optimal, DNA extractions for all the 602 samples were conducted with the DNeasy® Blood and Tissue Kit. The Quick-Start Protocol can be found at [www.qiagen.com/handbooks](http://www.qiagen.com/handbooks). Samples of muscle (25 mg) were incubated for one hour at 56°C with 180 µl Buffer ATL and 20 µl proteinase K. In separate repeated steps of spinning in the centrifuge for 1-3 minutes at 8,000 – 14,000 rpm, 200 µl Buffer AL, 200 µl ethanol, 500 µl Buffer AW1 and 500 µl Buffer AW2 were added to the sample with DNeasy Mini spin columns. The DNA was eluted in 100 µl Buffer AE. The DNA product was stored at -20°C until further use.

#### *Preparation of primers*

Initially, 3 microsatellites markers (Xg66, Xg144, Xg166) were chosen from Reeb *et al.* (2003) and 16 (A3, A4, A7, A8, A10, A113, A115, B6, B108, B112, C4, C7, C8, C10, D2B, D11) from Bradman *et al.* (2011), for this study. The potential multiplex groups of markers were arranged by considering the annealing temperature and size (bp) of the marker, and the fluorescent dye for the forward primer selected accordingly when ordering the primers (Blue- FAM, Yellow- NED, Red- PET or Green- VIC). The primers were resuspended in MilliQ water to create a 100 mM stock solution. Primers were placed on a shaker for 1-2 hours before storing at -20°C. The stock solutions were diluted into 10 mM working solutions.

#### *Primer optimisation*

The annealing temperatures of the markers as detailed by Reeb *et al.* (2003) and Bradman *et al.* (2011) were used to test the success of the PCR protocol on the available thermal cycler machines. One sample of relatively good DNA quantity (84 ng/µl) was used to test PCR amplification success through singleplex reactions. PCR amplification was performed in a volume of 10 µL with 50 ng of template DNA, 10 µM of each primer, 1 x GoTaq Flexi Buffer (Promega), 200 µM of dNTPs; 1 mM MgCl<sub>2</sub> and 0.5 units of *Taq* polymerase. The PCR was attained by a denaturation step of 5 minutes at 95°C, and continued with 35 cycles containing a 30 second denaturation segment at 95°C, a 45 second annealing segment at the optimum temperature, and a 30 second elongation segment at 72°C. The final elongation step was 10 minutes at 72°C.

### *Gel electrophoresis*

Success of single reaction PCR amplification at the aforementioned annealing temperatures was tested through agarose gel electrophoresis. A gel was made with 1x TBE buffer, 2% agarose powder and 1-2 µl ethidium bromide (for DNA staining) and placed in an electrophoresis bath containing 1x TBE buffer. Four microliters of PCR product and 2 µl of 5x DNA loading buffer were loaded onto the gel, with one lane dedicated to the 500 bp size standard, and run for 1 hour at ~120V until the dye front reached  $\frac{3}{4}$  of the total length of the gel. The gel was visualised by the ethidium bromide staining and UV-light exposure.

### *Capillary electrophoresis*

The PCR products were amplified separately and electrophoresed in four multiplex panels to test the success of electrophoresis on pooled PCR products. Capillary electrophoresis of PCR products with a GeneScan 600 LIZ® size standard (Applied Biosystems) was conducted on an ABI 3730xl DNA Analyzer at the Central Analytical Facilities, Stellenbosch University.

### *Allele scoring*

Allele scoring was conducted manually with the aid of Peak Scanner Software 2 (Applied Biosystems). Automatic binning of the alleles was performed with the program FLEXIBIN (Amos *et al.*, 2007).

### *DNA quantity thresholds for singleplex and multiplex PCR*

To determine the DNA quantity threshold for PCR success in multiplex reactions, groups of multiplex PCR reactions were tested with varying numbers of markers per reaction over a range of DNA quantities. The multiplex reactions were tested on samples of DNA quantity >20 ng/µl. The KAPA2G Fast Multiplex PCR Kit was used for multiplex reactions in a 10 µl reaction volume with 50 ng template DNA, 0.2 µM of each primer and 1X 2X KAPA2G Fast Multiplex Mix (containing 3 mM MgCl<sub>2</sub> at 1X).

Four multiplex reactions of 4- and 5-markers per reaction (three reactions with four markers each and one reaction with five markers = 19 markers) were tested on five samples ranging in DNA quantity of between 27 and 112 ng/μl (27, 37, 75, 84 and 112 ng/μl). Six multiplex groups of 2- and 3-markers per reaction (two reactions with two markers each and five reactions with three markers each = 19 markers) per reaction were tested on three samples ranging in DNA quantity of between 20 and 35 ng/μl (20, 26 and 35 ng/μl). The characteristics of the markers included in this study are listed in Table 3.

Table 3. Characteristics of the 19 microsatellite loci used in this study. Markers A10, A115, C4 were excluded from the study due to poor amplification success.

Locus	Motif sequence	Ta°C	Size range (bp)	Primers (5' - 3')
A3	(GACA)6	58	95-115	F: CAGTCGGGCGTCATCAAGTGAACCATCAGCGGCTCCT <sup>+</sup> R: GTTTCATCCTTGACTGGCACCTCCG
A4	(GACA)6	62	240-288	F: CAGTCGGGCGTCATCAGGGCAAGTAGATAACAGAATTA <sup>+</sup> R: GTTTCCTTAGCCCATCACCCAATCCATCGT
A7	(GACA)6	62	271-283	F: CAGTCGGGCGTCATCAAGCAGACTCTGAGCCAAGTGCAA <sup>+</sup> R: GTTTCCTTCATCACC AATCAGCCACC
A8	(GTCT)7	58	222-238	F: GTTTCCTTGCCCTTGCTGGAG <sup>+</sup> R: CAGTCGGGCGTCATCAGTGTTGGCAGGTGGTCTGGAG
A10	(CAGA)10	58	349-369	F: CAGTCGGGCGTCATCAGATTAAGGCAGCGGAGTCGAG <sup>+</sup> R: GTTTCCTTCGCTGGCAAGGCATTAGTTCAG
A113	(TCTG)6	54	212-226	F: GTTTCCTTCGCTGACAGACTTTACGACA <sup>+</sup> R: CAGTCGGGCGTCATCAATCAGCTTCCAGGACAACACA
A115	(ACAG)8	58	379-495	F: CAGTCGGGCGTCATCAGCAAATGTGTTTAGCCGGAGA <sup>+</sup> R: GTTTCCTTCCTGAATGGCAGTAATTGTG
B6	(GGAT)6	52	244-258	F: GTTTCCTGTGTACAGGATAACCGTCTTT <sup>+</sup> R: CAGTCGGGCGTCATCAAGGGCAGTCAATTAGGTAGGC
B108	(CCAT)13	58	168-226	F: CAGTCGGGCGTCATCATTAGTTTGTGGCAGTTATT <sup>+</sup> R: GTTTCCTTCATCCAGCCCTCCAATTATT
B112	(GATG)15	50	206-254	F: CAGTCGGGCGTCATCAGTTTATGTCAGCACAAGCACCT <sup>+</sup> R: GTTTCCTTCTGCAAGTTTCACCGTTTCTA
C4	(TAGA)12	56	448-500	F: GTTTCCTATCCGTCTCAGAGCAACTGGC <sup>+</sup> R: CAGTCGGGCGTCATCACTCTTAGTGACCCACGGGAAT
C7	(GATA)18	56	216-260	F: GTTTCGGAACGCACATGCAGAGCTTA <sup>+</sup> R: CAGTCGGGCGTCATCATTGGTCAAAGCTGCTCATATC
C8	(CTAT)22	58	152-236	F: CAGTCGGGCGTCATCACCTTCAATGTAGAGATGGCAGG <sup>+</sup> R: GTTTC AATGTGCGGTGGAGCTGTGGACAGA
C10	(CATA)14	52	194-270	F: CAGTCGGGCGTCATCAAATGGAGACTGCGATTAAGAT <sup>+</sup> R: GTTTCCTCAGTCTTCTGCCATAACTCA
D2B	(CAGT)8	58	157-185	F: CAGTCGGGCGTCATCAAAGCAACAACATTGTCTTCTG <sup>+</sup> R: GTTTC TGGCGTGAACGTGGCTCAATCC

D11	(TCAG)7	54	233-245	F: AGTCGGGCGTCATCAATGCAGGATTCCGCTGACCACT† R: GTTCTTTGGATGTGGATATACGGCACC
Xg66	(CA)11	52	140-150	F: TTTTCACCTTGTCACTGTGCG† R: ACAGACGTATACAACCACCTG
Xg166	(CAA)7	52	140-150	F: GTGAGTCATGTGTCACTGTGG† R: CCTCTGCCTGAAATACTTCAG
Xg144	(GGA)7	52	130	F: TTCCAATCATACCTCTGTCTATC† R: ACCACATCCATTATAGCATGTTG

†Indicates fluorescent primer

### *Sample filtering criteria*

A series of criteria were applied to remove individuals and markers that had poor amplification success. A threshold of 60% amplification success per individual was applied. All samples with 10 or more markers (from total of 16) that had amplified were kept in the dataset. Of these remaining samples, a threshold of 80% amplification success per marker was applied. All markers with more than 80% amplification success were kept in the dataset for the analyses.

### *Statistical analyses*

#### *Genetic diversity*

Data were organised in Microsoft Excel and prepared for input into the analysis software packages. Departures from neutrality of loci were tested using LOSITAN (Antão *et al.*, 2008) with 50,000 simulations, “neutral” mean  $F_{ST}$ , confidence intervals of 95% and a false discovery rate (FDR) of 0.1 with the infinite alleles model (IAM). LOSITAN detects loci under selection through an  $F_{ST}$ -outlier detection method when compared to calculated global  $F_{ST}$  values expected under neutrality.

Indices of genetic diversity were calculated in Arlequin 3.5.2.1. (Excoffier and Lischer, 2010), and included average number of alleles per locus ( $A$ ), observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ). The allele frequencies were calculated per locus and are included in Appendix B. To compare levels of polymorphism across loci, the polymorphic information content (PIC) of each marker for each of the two populations was estimated from observed allele frequencies (Botstein *et al.*, 1980). The PIC statistic is a function of both allele number and frequency and, therefore, is a better estimator of discriminatory power than is the number of alleles alone (Anderson and Karel, 2014). The PIC was calculated in CERVUS 3.0.7 (Kalinowski *et al.*, 2007). The allelic richness ( $R_s$ ),

which is a standardized index of the mean number of alleles per locus irrespective of sample size, was calculated with Fstat 2.9.3.2 (Goudet, 2001).

The coefficient of genetic differentiation ( $F_{ST}$ , among population variation), the inbreeding coefficient ( $F_{IS}$ , within population variation) and the overall fixation index ( $F_{IT}$ , total population variation) were estimated through the estimator of Weir and Cockerham (1984) using Genepop 4.2 (Raymond and Rousset, 1995) with numerical resampling by bootstrapping (1,000 times) and jack-knife procedures in order to estimate confidence intervals and the significance of the values. Positive  $F_{IS}$  values demonstrate an excess of homozygotes (positive correlation between homologous allele) or conversely, a deficiency of heterozygotes, relative to the Hardy-Weinberg model. This could be due to inbreeding, hence the label.  $F_{ST}$  values in the range of 0.0 to 0.05 may indicate little genetic differentiation, whereas values of  $F_{ST}$  above 0.25 indicate very great genetic differentiation (Wright, 1978; Hartl and Clark, 1997).

Departures from HWE and linkage disequilibrium (LD) were tested for each site at each locus using Genepop 4.2 (Raymond and Rousset, 1995) with 10,000 burn-in steps, and 500 batches of 5,000 Monte Carlo Markov Chain (MCMC) steps per batch. Statistical significance was assessed at each locus before and after sequential Bonferroni correction (Rice, 1989). In cases where observed genotype frequencies deviated significantly from HWE expectations, the program MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.*, 2004) was used to infer the most probable cause of the HWE departures: null alleles (Pemberton *et al.*, 1995), stuttering during the PCR amplification and large allele dropout (Wattier *et al.*, 1998). When a locus revealed evidence of null alleles, the frequency of null alleles ( $p_n$ ) was estimated by averaging estimations derived from three independent methods (Chakraborty *et al.*, 1992; Brookfield, 1996; Van Oosterhout *et al.*, 2006). The following population differentiation and population assignment tests were conducted on the four putative population scenarios (Figure 2).

### *Population differentiation*

Population differentiation was evaluated for each population scenario (i to iv) with pairwise  $F_{ST}$  tests executed in Arlequin 3.5.2.1 (Excoffier and Lischer, 2010) with 1,000 permutations and a significance level of 0.05. Fisher's exact test of genic (allele distribution) and genotypic (genotype distribution) distributions between pairs of populations in each scenario (i-iv) was conducted with Genepop 4.2 (Raymond and Rousset 1995; Rousset 2008) as an indication of population differentiation, with 1,

000 burn-in steps, and 100 batches of 1, 000 MCMC steps per batch. To assess variation within and among populations, a locus-by-locus and population specific analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) was conducted in Arlequin 3.5.2.1 (Excoffier and Lischer, 2010). The test was run among populations ( $F_{ST}$ ) and among individuals within populations ( $F_{IS}$ ) for each population scenario (i-iv). For all calculations, significance was assessed by 1,000 random permutations.

#### *Population assignment*

Genepop 4.2 (Raymond and Rousset, 1995) was used to test for isolation-by-distance (IBD) in Scenario II, the only scenario where levels of geographic distance is applicable with the East and West separated by the South, by plotting  $F_{ST} / (1 - F_{ST})$  (*i.e.* genetic distance) against the geographic distance between sampling sites, with a Mantel test (1,000 permutations of the data). This provided a one-tailed p-value for significance of the matrix correlation and a corresponding  $R^2$ . The Mantel test was carried out to examine whether the genetic distances between population pairs were linearly related to their geographical distances. The geographic position of each sampling block was specified as the coordinates at the mid-point of each sampling block, and  $F_{ST}$  values previously calculated in Genepop 4.2 were used.

First generation migrants, *i.e.* individuals born in a population other than the one in which they were sampled, were identified with GeneClass 2.0 (Paetkau *et al.*, 2004; Piry *et al.*, 2004) with a likelihood-based test statistic,  $L_{home}/L_{max}$ .  $L_{home}$ , the likelihood of finding a given individual in the population in which it was sampled, and  $L_{home}/L_{max}$ , the ratio of  $L_{home}$  to the greatest likelihood among all sampled populations. The Bayesian criterion of Rannala and Mountain (1997) in combination with the resampling method of Paetkau *et al.* (2004) was used to determine the critical value of the test statistic beyond which individuals were assumed to be migrants. Paetkau *et al.* (2004)'s probability computation of individual genotypes coming from each locality was calculated with a Markov chain (MC) resampling procedure by comparing individual genotypes to 1,000 simulated individuals per locality, and if the value was below  $P < 0.05$ , the individual was 'rejected' from that population. The frequency-based simulation method introduced by Paetkau *et al.* (2004) was selected as it is more representative of real population processes than other methods (*e.g.* Rannala and Mountain, 1997; Cornuet *et al.*, 1999) which have been shown to produce an inflated rate of type I errors (Paetkau *et al.*, 2004; Piry *et al.*, 2004). Nei's (1972) standard genetic distance ( $D_s$ ) for each population scenario was calculated in Genetix 4.05 (Belkhir *et al.*, 2004).



Three dimensional factorial correspondence analysis (3D-FCA) was performed with Genetix 4.05 (Belkhir *et al.*, 2004) to explore population divisions and relationships of swordfish, independent from a prior knowledge of their relationships. This analysis places all individuals in a hyperspace which has as many dimensions as there are alleles at different loci. The algorithm looks for independent (orthogonal) directions or eigenvectors in this hyperspace along which the inertia is maximum. The eigenvectors determine a series of axes and, by convention, the first axis is the one that has the highest contribution to the total inertia (Belkhir *et al.*, 2004).

A model-based Bayesian clustering algorithm was undertaken using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) to determine the number of genetic discrete populations ( $K$ ) with the highest posterior probability based on the microsatellite genotypes. The simulated  $K$  values ranged from 1 to 10. Twenty independent runs were implemented for each specific  $K$ -value in order to verify the consistency of the results. The simulations were conducted assuming an admixture model with correlated allele frequencies, which is considered as the superior model for detecting structure among closely related populations (Falush *et al.*, 2003; Hubisz *et al.*, 2009). MCMC consisted of 100,000 burn-in iterations followed by 100,000 iterations. STRUCTURE was selected because it performs well at low levels of population differentiation (Latch *et al.*, 2006). Structure Harvester (Earl and von Holdt, 2012) was used to determine the optimal number of clusters ( $K$ ), independent of the prior population allocation per individual, by obtaining the mean posterior probability of the data ( $L(K)$ ) and the  $\Delta K$  approach of Evanno *et al.* (2005). CLUMPP (Jakobsson and Rosenberg, 2007) aligned and averaged cluster assignments across replicate analyses from STRUCTURE utilising the greedy algorithm, while DISTRUCT 1.1 (Rosenberg, 2007) provided a visual representation of the aligned cluster assignments. Results from STRUCTURE were compared with the results from TESS 2.3.1. (Durand *et al.*, 2009).

TESS 2.3.1. (Durand *et al.*, 2009) implements Bayesian clustering for spatial population genetic studies, including simultaneous analysis from geographical data (Chen *et al.*, 2007). The method is based on a hierarchical mixture model where the prior distribution on admixture proportions (admixture model) is defined as a Hidden Gaussian Random Field (HGRF, admixture model) on a spatial individual network (tessellation). The program seeks population structure from individual multilocus genotypes sampled at distinct geographical locations without assuming predefined populations (Durand *et al.*, 2009). The conditional autoregressive (CAR) Gaussian admixture model was run in TESS, with the suggested burn-in period of 10,000 replicates and 50,000 MCMC iterations (Durand *et al.*, 2009) was run 20 times for each number of clusters from  $K_{\max} = 2$  to  $K_{\max} = 10$ . For

each value of  $K_{max}$ , the deviance information criterion (DIC) was computed. The ideal cluster number was chosen according to when the DIC values reached a plateau. The estimated admixture coefficients was averaged for the runs from the chosen  $K_{max}$  using the software CLUMPP. The resulting averaged Q matrix was visualized in DISTRUCT 1.1 (Rosenberg, 2007).

The TESS estimations of admixture proportions were displayed spatially with the tessplot function from “plot.admixture.r” ([http://membres-timc.imag.fr/Olivier.Francois/admix\\_display.html](http://membres-timc.imag.fr/Olivier.Francois/admix_display.html)) with the R packages *maps* and *fields* that interpolates expected admixture proportions on every point on a grid and displays it as a probability surface, known as universal kriging (Durand *et al.*, 2009).

### *Effective population size*

The effective population size ( $N_e$ ) is an essential parameter that informs about the sustainable management and conservation of exploited species (O’Leary *et al.*, 2013). It determines how vulnerable populations are to losing genetic diversity due to genetic drift, and consequently, it assesses their responsiveness and adaptation capabilities (Laconcha *et al.*, 2015). The software NEESTIMATOR 2.01 (Do *et al.*, 2014) was used to determine  $N_e$  with the Linkage Disequilibrium and the Heterozygote excess methods.

### *Marker power*

Finally, WHICHLOCI 1.0 was used to test the power of the markers to assign individuals to their current assigned populations by using allelic frequencies with 95% accuracy. An allele frequency differential was used following methods described in Shriver *et al.* (1997) to rank loci. The Whichloci (Banks *et al.*, 2003) method is a resampling technique that generates simulated populations from observed allele frequencies in experimental samples, and then assigns experimental individuals to populations on the basis of the likelihood of an individual’s genotype in each population. The software employs an empirical method for determining which combination of loci most likely provides a predefined population assignment power for individuals as well as statistical bounds on the performance of any particular group of loci. The log odds (LOD) level of assignment stringency was set at  $LOD = 1$ .

## **Results**

### *Comparison of DNA extraction methods*

The results of the four extraction methods tested on 10 samples (A to J) indicated that the 260/230 nm ratio was <0.7 for all extraction methods, possibly due to ethanol contamination in the samples. The CTAB extraction method had the highest DNA yield (average 247.9 ng/μl) and best average 260/280 nm ratio (1.97). The DNeasy® Blood and Tissue Kit achieved an average DNA yield of 26.6 ng/μl (± 20.77 ng/μl s.d.) and an average 260/280 nm ratio of 1.47. The SureFood® PREP kit had a higher average yield than the DNeasy® Blood and Tissue Kit (56.6 ng/μl) but with large deviation around the mean (115.2 ng/μl). The DNeasy® mericon Food Kit had the lowest DNA yield of 4.4 ng/μl.

The PCR success of markers A3 and A8 tested on three samples (F, G, J) extracted with the DNeasy Blood and Tissue kit are indicated in Figure 3. Sample J had the lowest DNA yield (18.1 ng/μl) and neither of the markers amplified. Sample F (24.06 ng/μl) and Sample G (84.38 ng/μl) amplified successfully. At this stage the minimum threshold for singleplex PCR success was assumed to be at around 20 ng/μl, and the DNeasy® Blood and Tissue Kit was deemed suitable for the project.

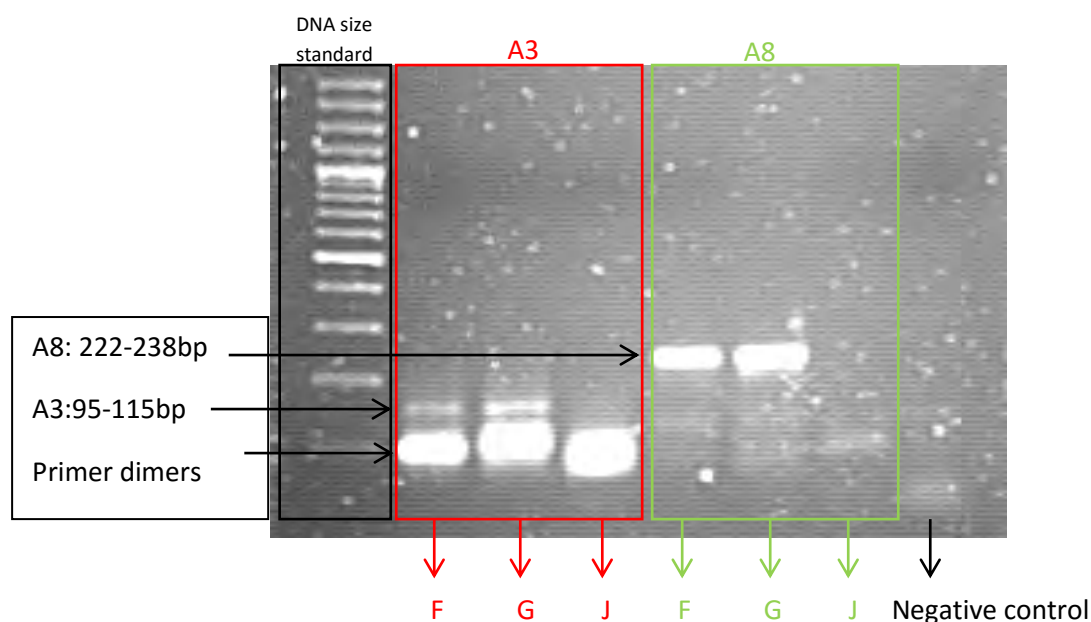


Figure 3. PCR of markers A3 and A8 on samples F, G and J extracted using the DNeasy® Blood and Tissue Kit. The 500bp DNA size standard is indicated.

#### *DNA quantity thresholds for singleplex and multiplex PCR*

The average DNA quantity of all samples was 35.73 ng/μl, average 260/280 nm ratio was 1.58 and the average 260/230 nm ratio was 0.53. The singleplex reactions had a success threshold of 20 ng/μl,

which removed 164 samples (27.7%) from the project that were  $<20$  ng/ $\mu$ l. Samples with  $>37$  ng/ $\mu$ l had the greatest 4- and 5-marker multiplex PCR amplification success based on genotyping results. A DNA threshold of multiplex reactions with 4- and 5-markers per reaction was set at DNA quantity  $>70$  ng/ $\mu$ l. The sample with  $>35$  ng/ $\mu$ l DNA amplified and genotyped successfully with 2- and 3-marker multiplex groups. Therefore, samples were divided into three sets of PCR reactions; 1) singleplex reactions with samples 20-34 ng/ $\mu$ l (180 samples, 41% of the remaining samples), 2) multiplex reactions of 2- or 3- markers per reaction on samples 35-69 ng/ $\mu$ l (205, 47% of the remaining samples), and 3) multiplex reactions of 4- or 5- markers per reaction on samples  $>70$  ng/ $\mu$ l (53, 12% of the remaining samples).

Through the process of optimising primers and the singleplex and multiplex PCR reactions, it was realised that the microsatellites A10, A115 and C4 (the three longest markers) consistently struggled to amplify with inconsistent amplification success with samples of highest DNA quantity. For this reason it was therefore decided to remove these three markers from the study. In general, the desiccated state of the muscle samples adversely affected the template DNA quantity and quality and overall amplification and genotyping success. Amplification was therefore successfully conducted on only 438 of the original 602 samples.

#### *Sample filtering criteria*

Once the filtering criteria had been applied per individual and per marker the final dataset consisted of 267 individuals (194 Indian Ocean ( $>20^{\circ}$ E) and 73 Atlantic Ocean ( $<20^{\circ}$ E)) (Figure 4) and 11 markers. The sex ratio was skewed with 181 females and 86 males in the dataset. The markers that were removed due to poor amplification success were A3, A4, C7, D11 and Xg66.

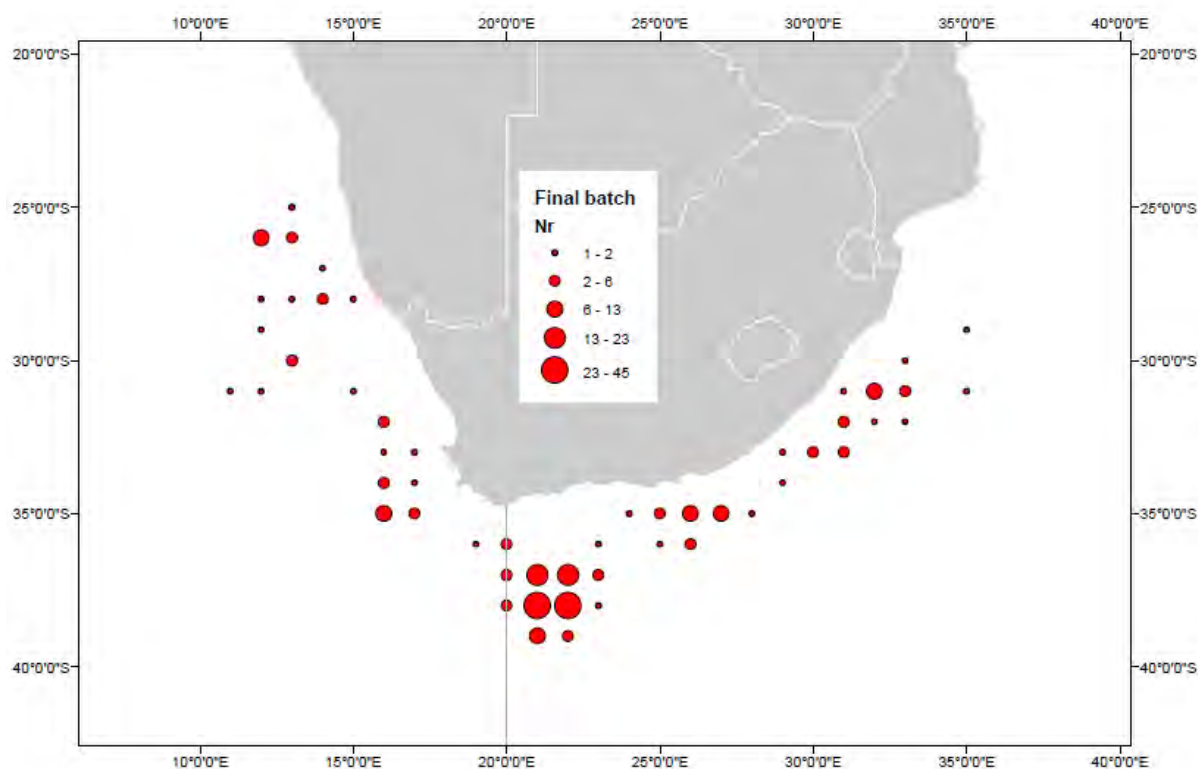


Figure 4. Geographic catch locations of individuals used in the final dataset.

### *Statistical analyses*

#### *Genetic diversity*

$F_{ST}$ -outlier analysis of the 11 loci on all individuals indicated that none of the loci were under selection (Figure 5). The 11 microsatellite loci varied in the number of alleles (5 – 25, mean = 11.4), observed heterozygosity (0.13 – 0.89, mean = 0.62) and expected heterozygosity (0.22 – 0.94, mean = 0.69) among populations (Table 4). The number of individuals genotyped in E20 ranged from 155 to 189 (median 176) and in W20 ranged from 57 to 69 (median 64). This was due, in part, to variation in the number of individuals collected from the two areas (Table 2) but may also be related to tissue degradation that prevented successful PCR amplification of both alleles in many individuals. Both populations had the same PIC value of 0.66. Allelic richness was 8.57 in the East and 8.31 in the West. Allelic frequencies of the 11 loci for E20 and W20 can be seen in Appendix B. The  $F_{ST}$  measure of -0.00001 is an indication of minimal to no detectable genetic differentiation between the two populations. The positive  $F_{IS}$  and  $F_{IT}$  values for all loci combined indicate heterozygosity deficiency in the total population (Table 5).

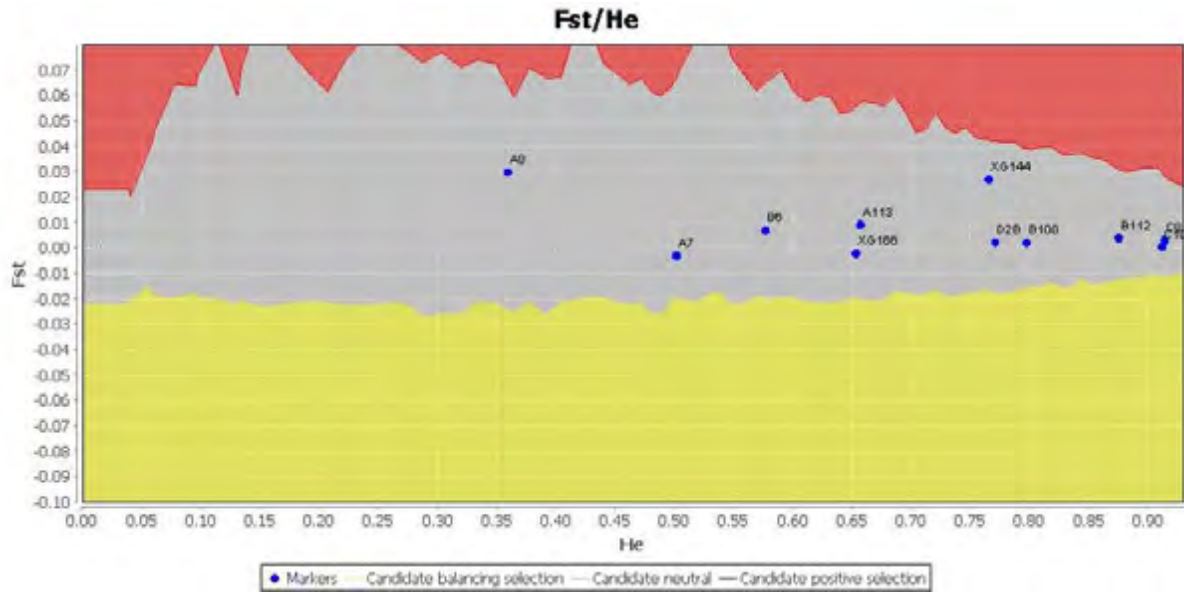


Figure 5. Identification of candidate loci under selection inferred from  $F_{ST}$  outlier analysis ( $P < 0.05$ ) of 11 microsatellite markers.

Table 4. Summary statistics for microsatellite DNA variability in swordfish. Number of individuals ( $N$ ), number of alleles ( $A$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), the Polymorphic Information Content ( $PIC$ ) and allelic richness ( $R_s$ ) was calculated for each locus per population (East ( $>20^\circ E$ ) and West ( $<20^\circ E$ )).

	$N$		$A$		$H_o$		$H_e$		$PIC$		$R_s$	
	E20	W20	E20	W20	E20	W20	E20	W20	E20	W20	E20	W20
A8	189	67	7	5	0.21	0.13	0.26	0.22	0.25	0.21	4.01	2.63
A7	176	62	7	5	0.43	0.39	0.48	0.43	0.45	0.40	4.68	4.75
A113	176	65	7	5	0.55	0.58	0.65	0.66	0.62	0.61	5.58	4.98
B6	155	62	7	6	0.56	0.65	0.55	0.60	0.50	0.54	5.94	5.00
B108	180	67	16	12	0.65	0.67	0.79	0.81	0.77	0.79	10.61	9.40
B112	177	61	13	12	0.77	0.79	0.87	0.88	0.86	0.85	9.15	9.60
C8	168	64	25	23	0.85	0.89	0.93	0.94	0.93	0.93	17.21	17.11
C10	163	61	20	20	0.82	0.84	0.91	0.92	0.90	0.91	14.35	18.24
D2B	181	69	11	7	0.71	0.68	0.76	0.76	0.72	0.72	7.10	5.00
Xg166	181	65	11	7	0.62	0.69	0.63	0.67	0.60	0.63	6.88	4.84
Xg144	158	57	15	10	0.59	0.63	0.74	0.77	0.70	0.73	8.80	9.89
Average			12.6	10.18	0.61	0.63	0.69	0.70			8.58	8.31

Table 5. Average Weir and Cockerman's F-statistics ( $F_{IS}$ ,  $F_{IT}$ ,  $F_{ST}$ ) per locus among populations, E20 and W20.

Locus	$F_{IS}$	$F_{ST}$	$F_{IT}$
A8	0.2461	0.0008	0.2467
A7	0.1108	-0.0026	0.1085
A113	0.1445	-0.0001	0.1444
B6	-0.0401	0.0014	-0.0386
B108	0.1806	-0.0002	0.1805
B112	0.1156	-0.0029	0.113
C8	0.0782	-0.0025	0.0759
C10	0.0976	-0.0007	0.097
D2B	0.0754	-0.0043	0.0714
Xg166	0.0085	0.0067	0.0151
Xg144	0.1972	0.0058	0.2019
All	0.1055	-0.0001	0.1054

Significant departures from HWE were observed in 14 of 22 single locus exact tests (Table 6) and all deviations except one (B6) were towards heterozygote deficiencies in either one or both of the populations (Table 4). Loci B6, B112, C8 and C10 were in HWE for the West and D2B and Xg166 for the East and West. Five (A8, A113, B108, C10, Xg144) of the nine loci that departed from HWE displayed null alleles and homozygote excess, and of those all except one locus (Xg144) underwent stuttering during PCR amplification (Table 7). These results indicate that true null alleles (*i.e.* alleles that failed to amplify because of base substitutions or deletions in PCR priming sites flanking microsatellite arrays) (O'Reilly *et al.*, 2004) could have been the cause of departures from HWE in only one of the nine loci. There was evidence of 16 of the 55 loci pairs with significant (Initial  $\alpha = 0.05$ ) LD before and after Bonferroni correction (Table 8). Of the loci pairs with significant LD, 13 included loci with null alleles (A8, A113, B108, C10, Xg144).

Table 6. Statistical significant departures from HWE per locus, p-value (initial  $\alpha=0.05$ ) was assessed before (\*) and after (\*\*) sequential Bonferroni correction.

Locus	E20	W20
A8	0.000**	0.000**
A7	0.000**	0.040*
A113	0.000**	0.013*
B6	0.001**	-
B108	0.000**	0.017*
B112	0.004*	-
C8	0.027*	-
C10	0.002*	-
D2B	-	-
Xg166	-	-
Xg144	0.000**	0.000**

Table 7. The results of MICRO-CHECKER testing for the presence of homozygote excess, stuttering during PCR amplification, large allele dropout and null alleles for the 11 loci from East ( $>20^\circ\text{E}$ ) and West ( $<20^\circ\text{E}$ ). na = not applicable.

Locus	Homozygote excess	Stuttering	Large allele dropout	Null alleles	Null frequency
A7	N	N	N	N	na
A8	Y	Y	N	Y	0.30
A113	Y	Y	N	Y	0.18
B6	N	N	N	N	na
B108	Y	Y	N	Y	0.22
B112	N	N	N	N	na
C8	N	N	N	N	na
C10	Y	Y	N	Y	0.20
D2B	N	N	N	N	na
XG144	Y	N	N	Y	0.21
XG166	N	N	N	N	na



Table 8. Loci pairs with significant linkage disequilibrium (LD), p-value (initial  $\alpha=0.05$ ) was assessed before (\*) and after (\*\*) sequential Bonferroni correction. Loci with null alleles are indicated with ^. S.E. = standard error.

Population	Locus #1	Locus #2	P-Value	S.E.
East (>20°E)	A8^	B6	0.000**	0.00
	A113^	B108^	0.021*	0.01
	A113^	C8	0.041*	0.02
	B6	C8	0.0165*	0.01
	B108^	C8	0.005*	0.01
	B108^	C10^	0.021*	0.01
	B112	C10^	0.049*	0.02
	C8	D2B	0.000**	0.00
	A113^	Xg166	0.035*	0.02
	C8	Xg166	0.037*	0.02
	Xg166	Xg144^	0.000**	0.00
West (<20°E)	A7	A113^	0.042*	0.01
	B108^	C8	0.027*	0.02
	B112	C10^	0.008*	0.00
	A7	Xg144^	0.046*	0.01
	C10^	Xg144^	0.024*	0.01

### *Population differentiation*

The joint null hypothesis of no heterogeneity between population pairs for any locus was rejected in 4 of 6 tests of genic differentiation (Table 9). Exact tests of genic differentiation between population pairs showed that significant heterogeneity was detected of the W17 and W20 populations with other locations. Pairwise  $F_{ST}$  indicated significant population structuring for scenario 2 between W17 and E30, and for scenario 4, between W30 and E30 (Table 10). AMOVA results indicated negligible heterogeneity between populations in every scenario with  $F_{ST}$  values between 0 and 0.007 (P between 0.45 and 0.94), likewise for genetic differentiation among individuals ( $F_{IS}$  between -0.04 and 0.018 (Table 11). Most of the time, negative variance component values indicate an absence of genetic structure (Schneider *et al.*, 2000). This result is concordant with the genotypic differentiation tests.

Table 9. Probability values of genic differentiation (lower diagonal) and genotypic differentiation (upper diagonal) tests for each population scenario. Significant p-values (<0.05) is indicated by an asterisk \*.

		Scenario 1		Scenario 2			Scenario 3		Scenario 4	
		East (>20°E)	West (<20°E)	East (>30°E)	South (17-30°E)	West (<17°E)	East (>17°E)	West (<17°E)	East (>30°E)	West (<30°E)
Scenario 1	East (>20°E)	-	0.13							
	West (<20°E)	0.01*	-							
Scenario 2	East (>30°E)			-	0.21	0.05				
	South (17-30°E)			0.17	-	0.18				
	West (<17°E)			0.02*	0.04*	-				
Scenario 3	East (>17°E)						-	0.15		
	West (<17°E)						0.02*	-		
Scenario 4	East (>30°E)								-	0.09
	West (<30°E)								0.08	-

Table 10. Pairwise  $F_{ST}$  (lower diagonal) and corresponding significance values (upper diagonal) (\* $p < 0.05$ ) for the four population scenarios.

		Scenario 1		Scenario 2			Scenario 3		Scenario 4	
		East (>20°E)	West (<20°E)	East (>30°E)	South (17-30°E)	West (<17°E)	East (>17°E)	West (<17°E)	East (>30°E)	West (<30°E)
Scenario 1	East (>20°E)	-	0.51							
	West (<20°E)	-0.0003	-							
Scenario 2	East (>30°E)			-	0.12	0.04*				
	South (17-30°E)			0.00373	-	0.54				
	West (<17°E)			0.0067	-0.0004	-				
Scenario 3	East (>17°E)						-	0.49		
	West (<17°E)						0.00004	-		
Scenario 4	East (>30°E)								-	0.03*
	West (<30°E)								0.00558	-

Table 11. Analysis of molecular variance (AMOVA) among populations for the four population scenarios.

	Genetic Structure	Variance component	% of total	Fixation index
Scenario 1	Among populations	-0.00074	-0.02	Fst = 0.000 (P = 0.98)
	Among individuals within populations	-0.03429	-1. 16	Fis = 0.018 (P = 0.85)
	Within individuals	2.99813	101. 18	Fit = 0.008 (P = 0.86)
Scenario 2	Among populations	0.00442	0. 15	Fst = 0.00149 (P = 0.94)
	Among individuals within populations	-0.03675	-1. 24	Fis = -0.01241 (P = 0.87)
	Within individuals	2.99813	101.09	Fit = -0.01090 (P = 0.85)
Scenario 3	Among populations	0.0003	0.01	Fst = 0.00010 (P = 0.95)
	Among individuals within populations	-0.03469	-1. 17	Fis = 0.00010 (P = 0.88)
	Within individuals	2.99813	101. 16	Fit = -0.01160 (P = 0.86)
Scenario 4	Among populations	0.01703	0.57	Fst = 0.00572 (P = 0.45)
	Among individuals within populations	-0.03759	-1. 26	Fis = -0.01270 (P = 0.89)
	Within individuals	2.99813	100.69	Fit = -0.00691 (P = 0.87)

#### Population assignment

Nei's (1972) Standard Distance ( $D_s$ ) was calculated per scenario and resulted in the largest genetic distance between the populations of scenario 3, W17 and E17, and scenario 2, W17 and E30 (Table 12). The Mantel regression test indicated a lack of correlation between genetic divergence and geographic distance ( $R^2 = 0.2969$ ,  $P = 0.54$ ) across populations in scenario 2 (Figure 6). The rate at which individuals correctly assign to their sampled locality can also be used as an assessment of population genetic structure (Manel *et al.*, 2005). Scenarios 1 and 3 had the highest percentage of migrants (8.23% and 6.37%, respectively), and the misassignments (*i.e.* migrants) were individuals that had originated in neighbouring East ( $>17^\circ\text{E}$  and  $>20^\circ\text{E}$ ) populations and were caught in the West ( $<17^\circ\text{E}$ ) (Table 13). A summary across population scenarios indicates that on average 5.8% of migrants originating from the South and East were caught in the West, 1.5% of migrants originating from the South were caught in the West and East, and 1.62% of migrants from the West and South were caught in the East.

Three dimensional factorial correspondence analyses (3D-FCA) explained 100.00% of the overall variation in scenarios 1, 3 and 4, and 54.45% of the variation in scenario 2, with additional separation noticeable on the second axis. Every scenario displayed considerable overlap of individuals from each population, and the boundary between proposed populations was not clear, with admixture zones present (Figure 7).

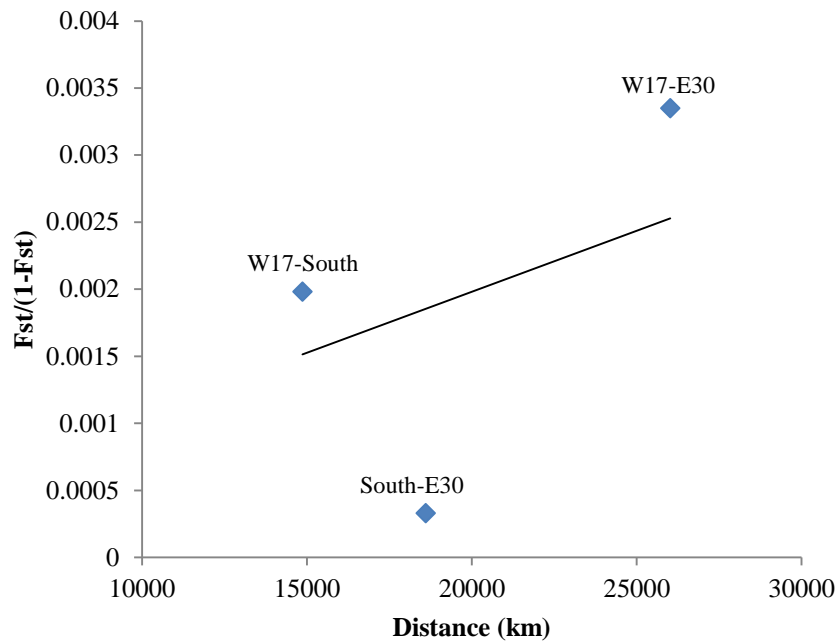


Figure 6. Isolation-by-distance (IBD) plotting the genetic distance ( $F_{ST} / (1 - F_{ST})$ ) against the geographic distance (km) for E30, South and W17.

Table 12. Nei's (1972) genetic distance  $D_s$  for scenarios 1 to 4.

		Scenario 1	Scenario 2		Scenario 3	Scenario 4
		East (>20°E)	South (17-30°E)	East (>30°E)	East (>17°E)	East (>30°E)
Scenario 1	West (<20°E)	0.013	0.033		0.044	0.036
Scenario 2	East (>30°E)					
	West (<17°E)	0.016	0.044			
Scenario 3	West (<17°E)	0.044				
Scenario 4	West (<30°E)					

Table 13. The results of the detection of migrants tests conducted in GeneClass 2.0, for each population scenario.

		Correct population		
Scenario 1	Assigned population	West (<20°E)	East (>20°E)	
	West (<20°E)		17	
	East (>20°E)	5		
	Total number of migrants:	22		
	Percentage migrants:	8.23%		
Scenario 2	Assigned population	West (<17°E)	South (17-30°E)	East (>30°E)
	West (<17°E)		2	0
	South (17-30°E)	1		0
	East (>30°E)	1	6	
	Total number of migrants:	10		
	Percentage migrants:	3.75%		
Scenario 3	Assigned population	West (<17°E)	East (>17°E)	
	West (<17°E)		14	
	East (>17°E)	3		
	Total number of migrants:	17		
	Percentage migrants:	6.37%		
Scenario 4	Assigned population	West (<30°E)	East (>30°E)	
	West (<30°E)		0	
	East (>30°E)	12		
	Total number of migrants:	12		
	Percentage migrants:	4.49%		

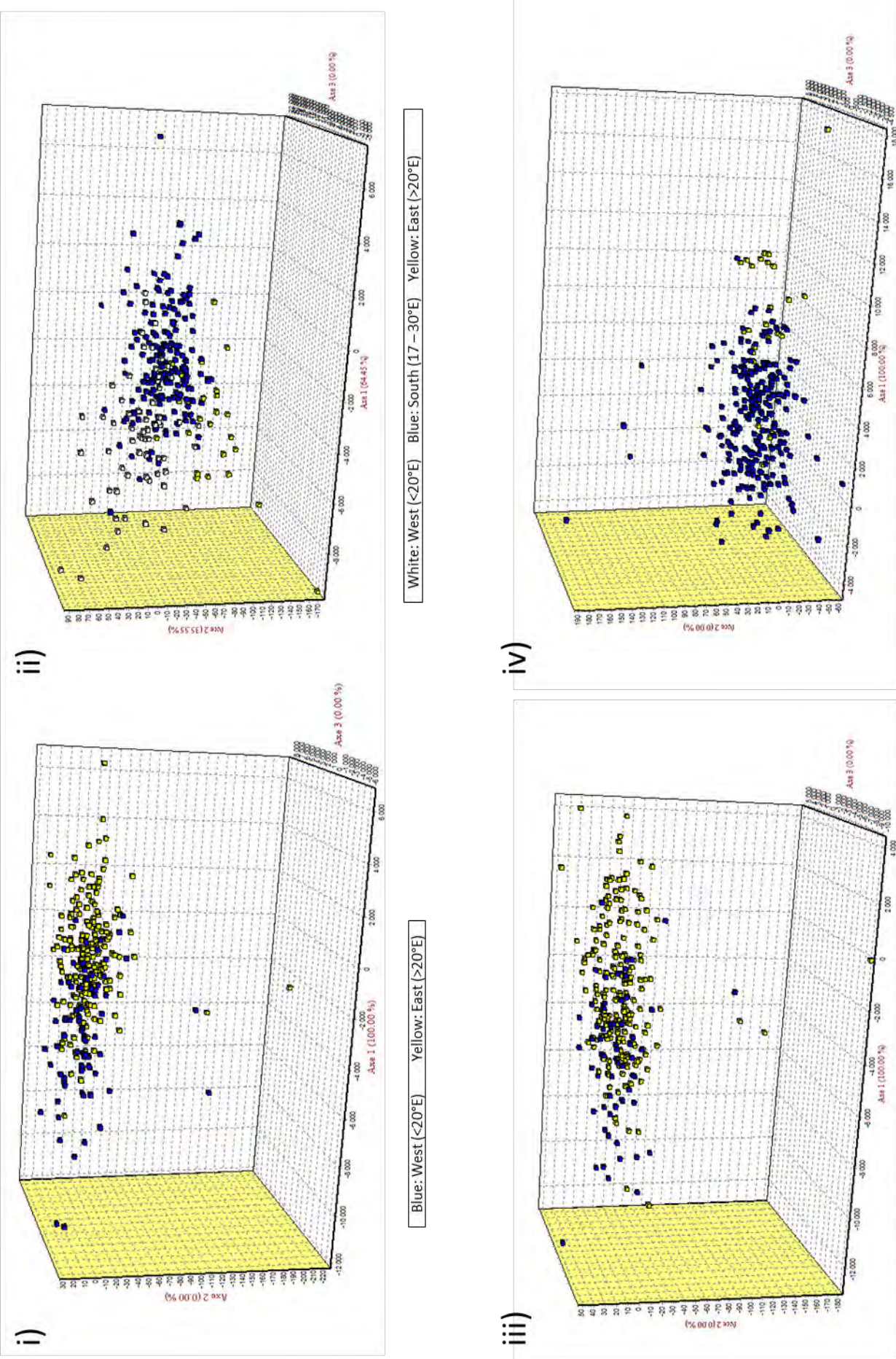


Figure 7. i-iv) Factorial correspondence analysis (FCA) in three dimensions of the four population scenarios 1 to 4 (i-iv, respectively).

STRUCTURE performs well at low levels of population differentiation (Latch *et al.* 2006) and may be able to detect structure in this data if it is present. Calculation of  $\Delta K$ , a measure of the second order rate of change in the likelihood of  $K$  (Evanno *et al.*, 2005), from the STRUCTURE output produced a modal value of the statistic at  $K = 6$  (Figure 8a). While the largest value of  $\Delta K$  was at  $K = 4$ , a second mode was present at  $K = 6$ . In cases where STRUCTURE finds clustering solutions with similar probabilities at different values of  $K$ , the lowest value is typically the most accurate (Pritchard *et al.*, 2000; Pritchard and Wen, 2004). The expectation was two clusters of swordfish since there are two recognised stocks, the South Atlantic and the Indian Ocean.

While Evanno's  $\Delta K$  method seeks to detect the uppermost hierarchical level of population structure, the method is less reliable at lower levels of genetic differentiation and may incorrectly estimate  $K$  (Waples and Gaggiotti, 2006). Therefore, the estimation of  $K$  using the *ad hoc* evaluation of mean posterior probabilities from multiple analyses of  $K$ , *i.e.*  $L(K)$  (Pritchard *et al.*, 2000), appears to be more appropriate in this instance than the  $\Delta K$  approach (Evanno *et al.*, 2005). The mean posterior probabilities of  $L(K)$  indicated  $K = 4$  (Figure 8b).

Genetic clustering between W20 and E20 was not visually detected in the Bayesian clustering analysis averaged in CLUMPP and displayed by DISTRUCT for  $K = 2, 3$  or 4 (Figure 9). The proportion clustering membership ( $Q$ ) did not produce patterns concordant with the geographic boundary. In  $K = 2$ , individuals with majority cluster 1 (yellow)  $Q$  are found across the geographic locations.

The model-based clustering results in TESS indicated the existence of four clusters based on the lowest DIC value (Figure 10). Average membership coefficients of each individual to either  $K=2, 3$  or 4 from TESS analyses is displayed in Figure 11. As with the results from STRUCTURE, there are no clear memberships of individuals to either of the two populations. A posterior predictive map of the admixture proportions for  $K_{max} = 4$  (Figure 12) was generated from the spatial interpolation (kriging) procedure. The  $K_{max} = 4$  may indicate sublevels of structuring. When considering  $K_{max} = 4$  the red and green clusters (or admixture proportions) distribute together, and the blue and yellow clusters are neighbouring and distribute together. Therefore, a weak differentiation boundary may be present in the region of 27°E. A posterior predictive map of the admixture proportions for  $K_{max} = 2$  was included to visualise the clustering without the potential sublevels of structuring mentioned by Evanno *et al.* (2005) (Figure 13). At this level of clustering an admixture zone between 14°E and 27°E may exist. A graphical summary of the population differentiation and population assignments tests are indicated in Figure 14.

### *Effective population size*

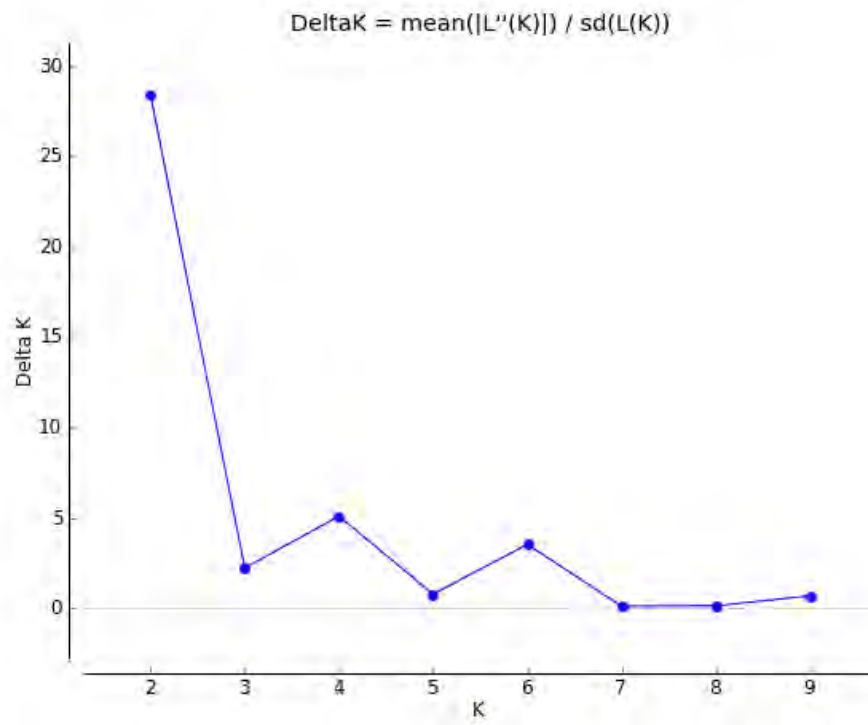
The results from the Linkage Disequilibrium method of determining  $N_e$  (NEESTIMATOR) produced an alarmingly small value of  $N_e = 337$  in the East ( $>20^\circ\text{E}$ ), with wide 95% confidence intervals of between 48.9 and infinity, and  $N_e = 1821.1$  in the West ( $<20^\circ\text{E}$ ), with wide 95% confidence intervals of between 438.3 and infinity. Similarly, the Heterozygote Excess method determined the effective population size to equal infinity in both populations. These results indicate that the data is not informative enough to determine  $N_e$ .

### *Marker performance*

The results from WHICHLOCI indicated that with a stringency of  $\text{LOD} = 1$ , neither the 11 loci combined nor 6 loci (markers with null alleles removed) combined were sufficient to assign the individuals to the current population assignment of W20 and E20 with 95% confidence.



a)



b)

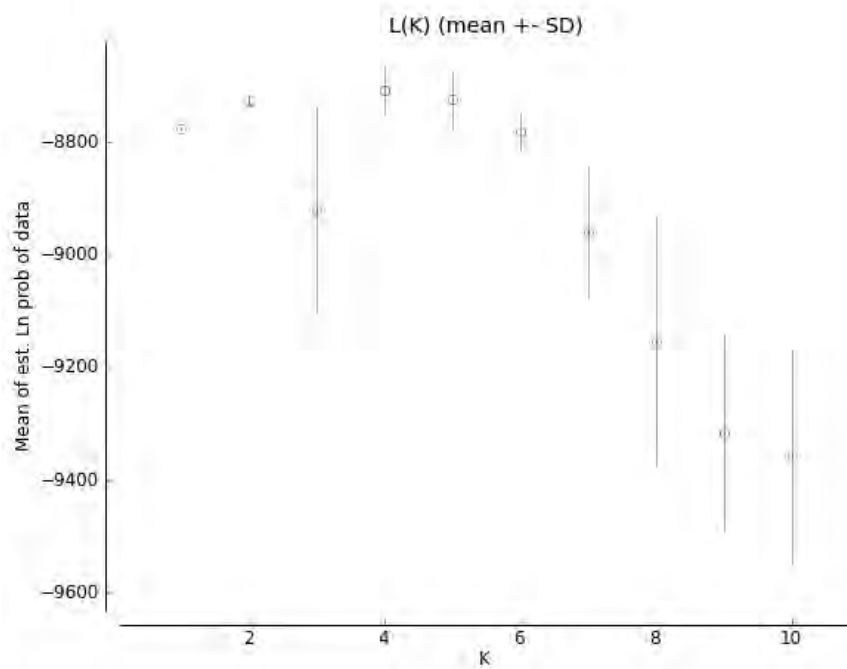


Figure 8. Assignment of swordfish to populations by the STRUCTURE program. The peak of (a) Evanno's delta k ( $\Delta K$ ) and (b) the mean log likelihood of the data  $[L(K)]$  represents the most likely number of subpopulations.

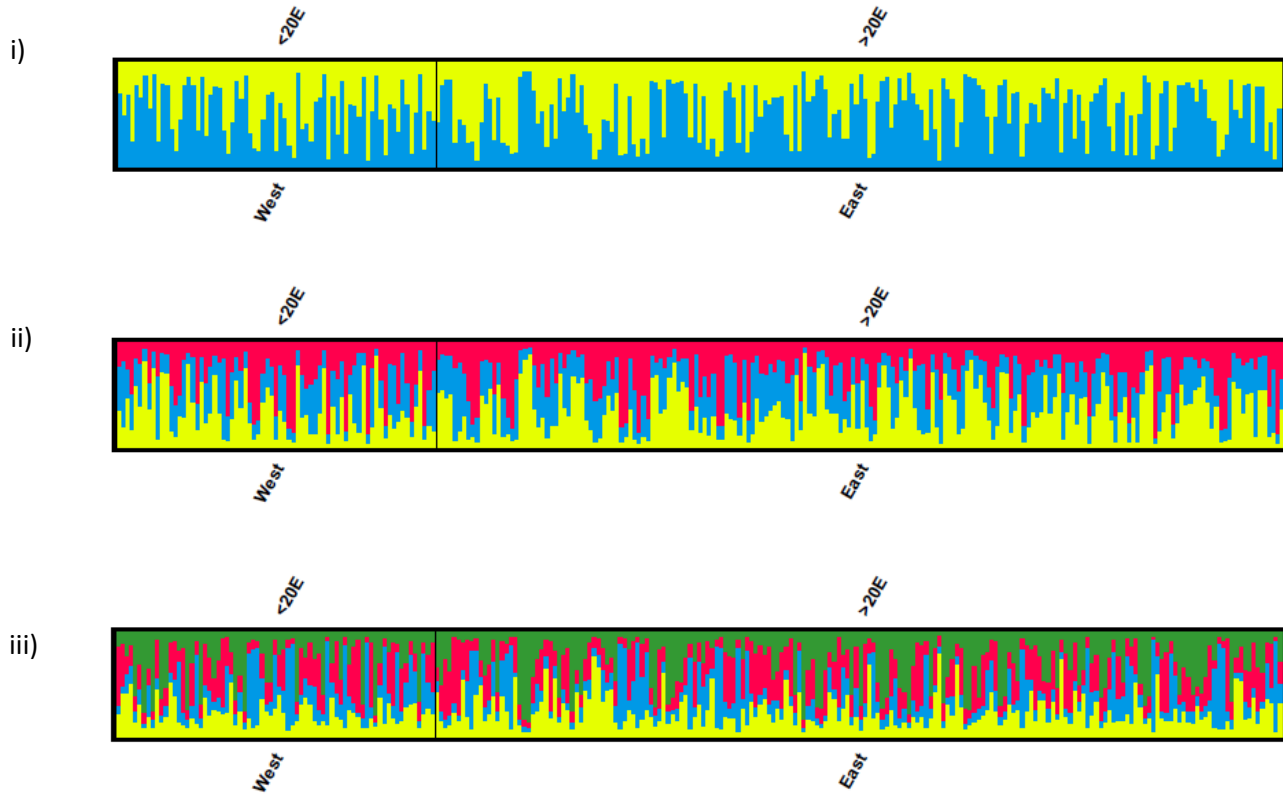


Figure 9. Bayesian clustering results inferred by STRUCTURE for three scenarios of  $K$ , i)  $K = 2$ , ii)  $K = 3$ , iii)  $K = 4$

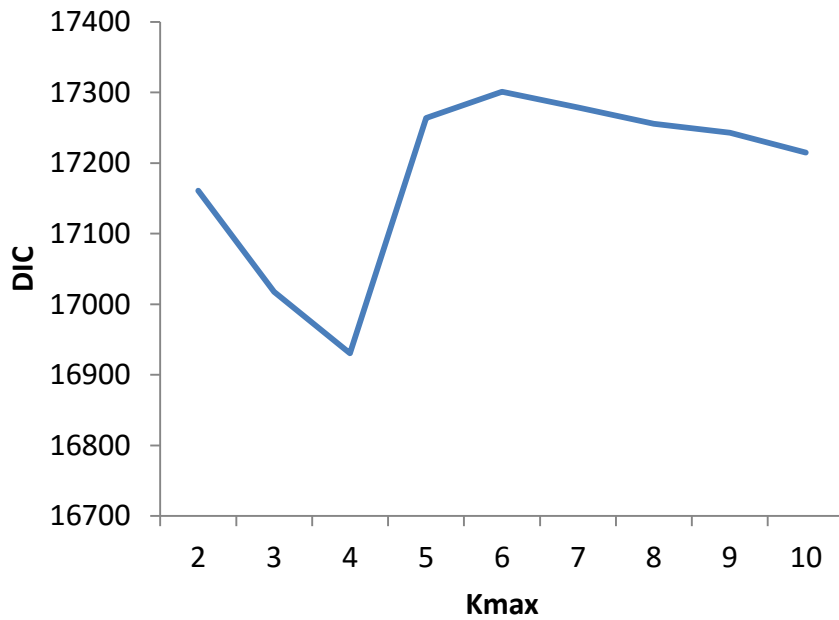


Figure 10. The deviance information criterion (DIC) for 20 TESS runs with  $K_{\max}$  ranging from 2 to 10.

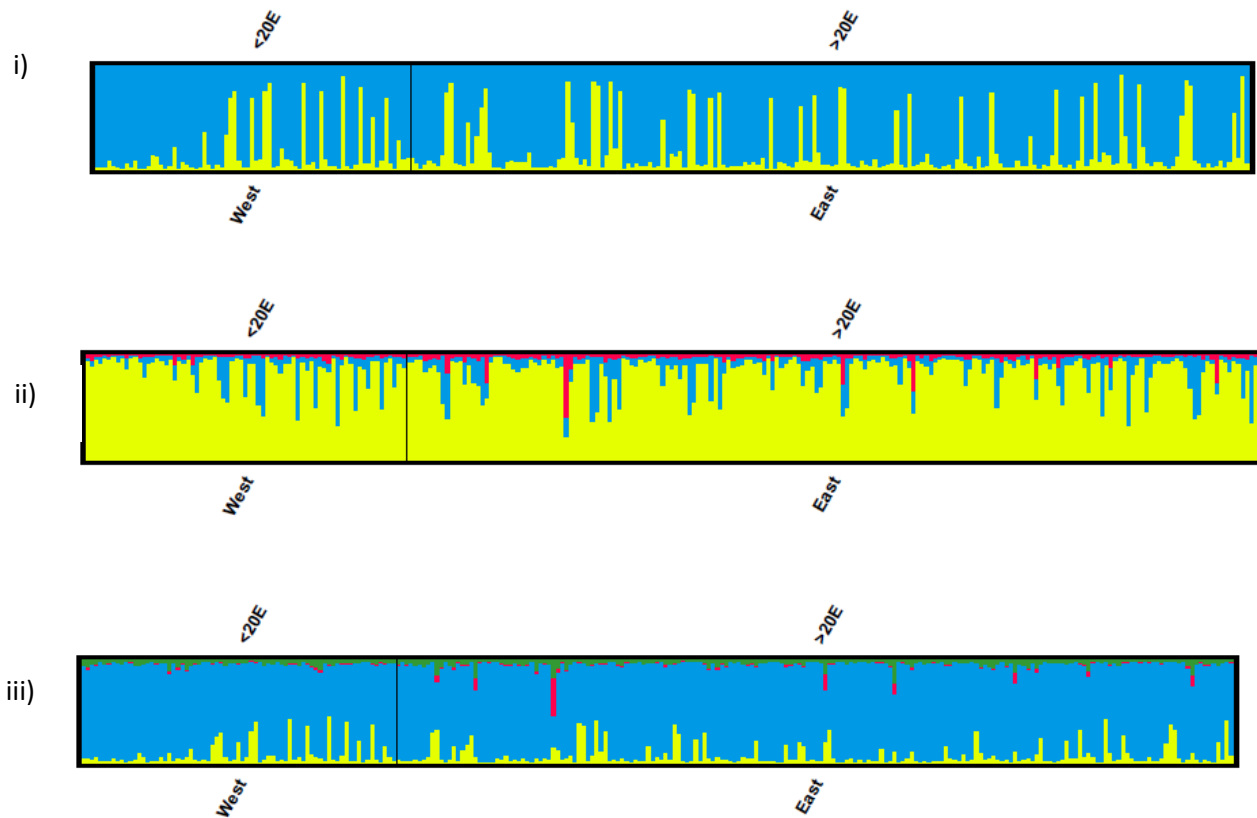


Figure 11. Bar plots representing admixture proportions for swordfish from a spatial assignment test performed in TESS 2.3.1. for i)  $K = 2$ , ii)  $K = 3$ , and iii)  $K = 4$ .

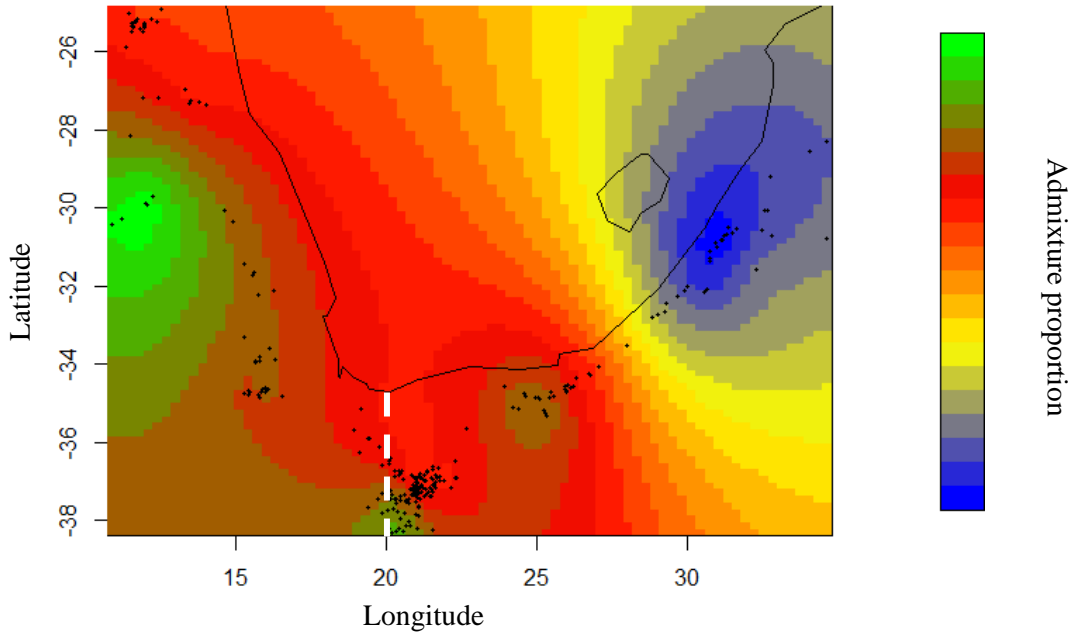


Figure 12. Posterior predictive map of the admixture proportions for  $K_{\max} = 4$  clusters generated from the spatial interpolation (kriging) procedure implemented in TESS 2.3.1. Each colour (green, red, yellow, blue) indicates one of the four clusters. The current management boundary at 20°E is indicated.

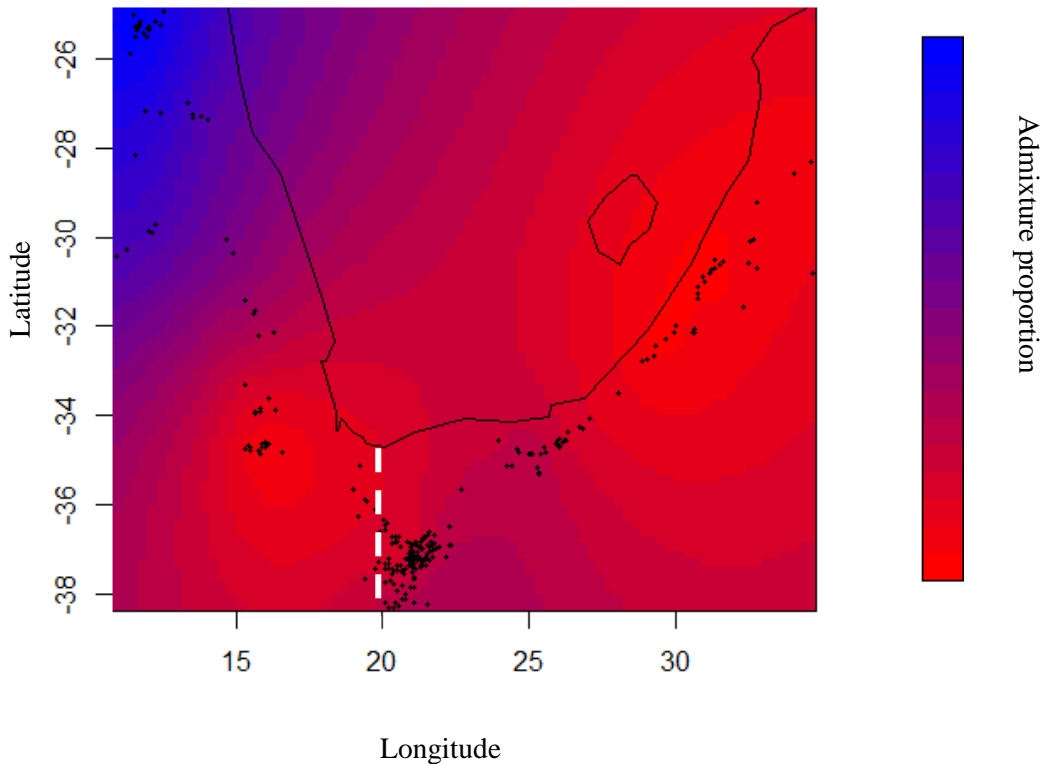


Figure 13. Posterior predictive map of the admixture proportions for  $K_{\max} = 2$  clusters generated from the spatial interpolation (kriging) procedure implemented in TESS 2.3.1. Each colour (blue and red) indicates one of the two clusters. The current management boundary at 20°E is indicated.

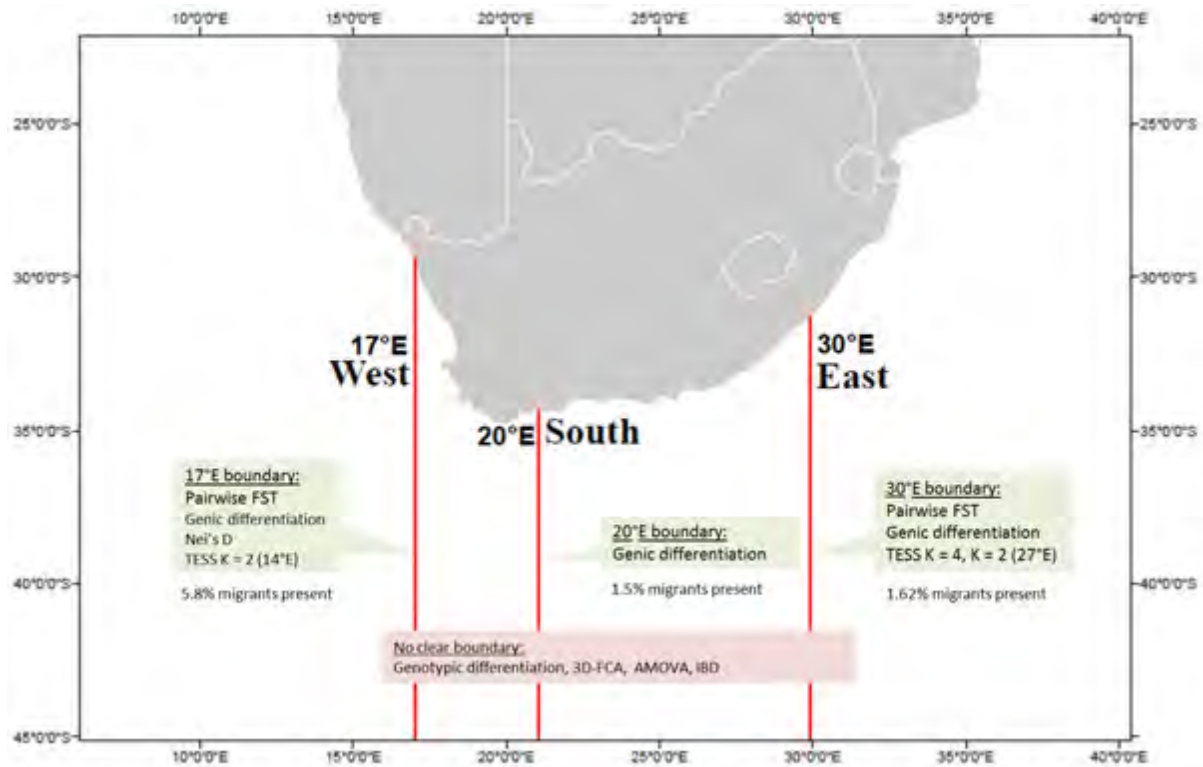


Figure 14. A graphic summary of the tests conducted on swordfish in this study.

## Discussion

Weak structure was detected between the two managerial swordfish populations (West <20°E and East >20°E) sampled in a regional geographic scale around South Africa. This is not surprising since active dispersal of adult swordfish of the two recognised stocks results in mixing in this area, in addition to genetic homogenisation due to passive drift of larvae.

In the marine environment many studies have failed to detect statistically significant population structuring because of low differentiation, especially over small geographical distances (*e.g.* in cod, Árnason *et al.*, 1992; Gjøsæter *et al.*, 1992). Low levels of differentiation in marine organisms are most likely due to extensive gene flow (Ward *et al.*, 1994; Waples, 1998; Avise, 2000) and do not necessarily imply that structuring does not exist, but that more powerful means are required to detect them (Knutsen *et al.*, 2003). Marine organisms, even if weakly differentiated on a small geographical scale, often show evidence of differentiation over larger distances, probably because the long distance acts as an isolation mechanism (Knutsen *et al.*, 2003).

Despite the weak structure of swordfish in this study, the results corroborate previous findings of population differentiation in swordfish between the Indian and Atlantic oceans that were sampled at larger geographical scales than this study (Chow *et al.*, 1997; Alvarado Bremer *et al.*, 1999; Chow and Takeyama, 2000; Kotoulas *et al.*, 2006; Alvarado Bremer *et al.*, 2007; Muths *et al.*, 2013). Previous evidence of weak differentiation between the Indian and Atlantic oceans with microsatellites includes studies by Ward *et al.* (1997), Alvarado Bremer *et al.* (1998); Chow *et al.* (2000), Graves and McDowell (2003); Durand *et al.* (2005), Chiang *et al.* (2008), Albaina *et al.* (2013) and Laconcha *et al.* (2015).

The low numbers of individuals successfully genotyped per area, 94 from W20 and 173 from E20, may have contributed to some loss of power. Nevertheless, Ruzzante (1998) showed that samples of 50 or greater are sufficient to produce relatively precise estimates of  $F_{ST}$  with highly variable microsatellites (O'Reilly *et al.*, 2004). Reeb *et al.* (2003) indicated that the markers Xg66, Xg144 and Xg166 displayed significant divergence between two populations (Ecuador and Mediterranean), and observed heterozygosity of between 0.15 and 0.960, deeming them suitable for population genetics studies. These three markers were also utilised in the study by Ward *et al.* (2001) on the population structure of Australian swordfish, with two markers (Xg66 and Xg144) providing the highest gene differentiation values. However, a study by Kasapidis *et al.* (2008) on the stock structure of swordfish in the Pacific revealed Xg144 to be least polymorphic. The 16 markers chosen from Bradman *et al.* (2011) (A3, A4, A7, A8, A10, A113, A115, B6, B108, B112, C4, C7, C8, C10, D2B, D11) were deemed suitable for population structure studies on swordfish; however, four of the markers (B6, B108, B112, C4) did not meet the HWE, similarly to the current study.

O'Reilly *et al.* (2004) suggested that null alleles may be common in large marine populations that have increased sequence heterogeneity, attributed to increased effective population sizes and reduced loss of variation due to drift. However, the use of degraded tissue samples provided low quality DNA which may have resulted in unsuccessful amplification and had a direct impact on the degree of null allele markers. This has impacted the departures from HWE and LD since five of the nine markers not in HWE had null alleles and 13 of the 16 pairs of markers in LD were markers with null alleles. In addition to the presence of null alleles, the departure from HWE, common in marine fish (O'Connell and Wright, 1997; Karlsson and Mork, 2005), can be explained by factors such as inbreeding, the Wahlund effect, or selection (Wittke-Thompson *et al.*, 2005).

The positive  $F_{IS}$  and  $F_{IT}$  values reflect homozygote excess and/or null alleles in the markers. Of the extraction methods tested, the CTAB protocol provided the highest DNA yield. It is recommended that for future studies on degraded tissue samples, the time is dedicated to the CTAB extraction protocol to maximise the success of the amplification and genotyping procedures that follow.

The  $F_{ST}$  values observed in this study were too low to indicate even a weak signal of genetic differentiation. Similarly, the AMOVA results among populations and individuals, and genotypic differentiation tests did not reveal population differentiation in any of the scenarios. On this regional geographical scale, the pairwise  $F_{ST}$  values were less than 0.05, the level that indicates little genetic differentiation. This level of weak differentiation is similar in magnitude to those reported for other marine fish species with potentially high levels of gene flow (e.g. Elliot and Ward, 1992 (orange roughy *Hoplostethus atlanticus*); Gold *et al.*, 1994 (red drum *Sciaenops ocellatus*); Bentzen *et al.*, 1996 (Atlantic cod *Gadus morhua*); Borsa *et al.*, 1997 (flounders); Ruzzante *et al.*, 1999 (cod *Gadus* spp.); Shaw *et al.*, 1999 (Atlantic herring *Clupea harengus*); Lundy *et al.*, 2000 (European hake *Merluccius merluccius*); Nesbø *et al.*, 2000 (Atlantic mackerel *Scomber scombrus*); De Innocentiis *et al.*, 2001 (Dusky grouper *Epinephelus marginatus*); McPherson *et al.*, 2001 (Atlantic herring *Clupea harengus*); Wirth and Bernatchez, 2001 (European eel *Anguilla anguilla*); Withler *et al.*, 2001 (Pacific Ocean perch *Sebastes alutus*); Knutsen *et al.*, 2003 (Atlantic cod *Gadus morhua*)).

Despite the low pairwise  $F_{ST}$  values, significant differentiation was present between W17/E30 (Scenario 2) and between W30/E30 (Scenario 4) (Table 10). The genic differentiation tests built on this result and included significant differentiation of W17/S (Scenario 2), W17/E30 (Scenario 2), W17/E17 (Scenario 3), W20/E20 (Scenario 1) (Table 9). These results indicate variation in the interactions between the populations, supporting scenarios of individuals moving from the Atlantic stock (<20°E) into the Indian Ocean area (>20°E) and *vice versa*; a result in concordance with Muths *et al.* (2013). Because the level of differentiation was quite low, it may at first be dismissed as not being biologically meaningful (c.f. Waples 1998; but see Wirth and Bernatchez, 2001). Knutsen *et al.* (2003) concludes that this is unwarranted; any statistically significant difference in allele frequency, no matter how small, indicates that the samples are from separate statistical populations.

There was additional evidence for swordfish distributing across the management boundary through the presence of first generation migrants that originated from E17 and South caught in the W17 region, and migrants that originated from W17 and South caught in the E17 region (Figure 14). The assignment tests, however, indicated that the proportion of migrants between the two populations

was very low. When the largest Nei's genetic distance ( $D$ ) values are considered (0.044 and 0.036) then an indication of population differentiation exists and the Indian Ocean stock distribution extends over the management boundary at 20°E.

The 3D-FCA for all scenarios revealed extensive overlap of individuals between the populations for all scenarios. There was no clear boundary between populations, indicating a large degree of admixture. It was not surprising then that the population structure did not follow an isolation-by-distance pattern since the West, South and East regions are relatively in close proximity. As the species is wide ranging, migratory and potentially admixed, the geographical range may be too restricted to detect a relationship of genetic distance with geographic distance. Other studies have reported an IBD distance pattern of geographical distances between samplings sites in excess of 1,000 km (*e.g.* Mork *et al.*, 1985; Pogson *et al.*, 1995, 2001).

The estimated number of clusters ( $K_{max} = 4$ ) generated by STRUTCURE and TESS does not indicate structure in the bar plots of individual admixture proportions across the geographic range. Although results from STRUCTURE and TESS analysis suggest that swordfish form four genetic clusters, it can be argued that two clusters are more likely (Barker *et al.*, 2015). Hubisz *et al.* (2009) cautioned that STRUCTURE can overestimate the number of clusters, for example when there is inbreeding or relatedness among some individuals. Moreover, the number of clusters is not well-defined in settings where the allele frequencies vary smoothly across the landscape (Wasser *et al.*, 2004). The additional  $K = 2$  bar plots differ between STRUCTURE and TESS outputs yet both indicate the possibility of admixture between the populations, since the proportions of individuals that have dominant cluster 1 (yellow) or cluster 2 (blue) are distributed across the geographic range. The boundaries suggested from the posterior predictive maps of admixture proportions for  $K = 4$  (Figure 12) and  $K = 2$  (Figure 13) are independent of any suggested boundaries created per scenario (17°E, 20°E, 30°E) yet the map indicates degrees of admixture in the South with a separation between the Atlantic and Indian oceans in the West and East between 14°E and 27°E.

The insufficiency of the 11 loci to allocate individuals into the current W20 and E20 populations, according to WHICHLOCI, could be attributed to few markers utilised once the markers with null alleles were removed.

Alluded to in the literature review, a large effective population size that limits genetic drift can be one of the causes of HWE and genetic homogeneity. The effective population size is the number of



individuals in a population who contribute offspring to the next generation. In this study the data was insufficient to determine the effective population size, and its relation to the census population size (*i.e.* estimated total biomass) of the two stocks. The effective population size may, however, be one of the causes of weak differentiation in this area.

Since the area of the Panama Canal was sealed off about 3.5 million years ago, the water exchange between the tropical Atlantic Ocean and eastern Pacific Ocean was cut off (Keigwin 1982; Coates *et al.*, 1992; Li *et al.*, 2015). The presence of genetic differentiation between Atlantic Ocean and Pacific Ocean stocks of large pelagic species is expected since the connection between these two oceans lies at 56°S, far below the geographic range of tunas and billfish. On the other hand, the southern African region, separating the Indian and Atlantic oceans, is in the 30-40°S latitudinal range, an area suitable for tropical and temperate tunas and for billfish to inhabit. Weak differentiation at regional geographical scales in this area is therefore not surprising as these species have wide environmental parameter limits that extend across this area.

Ocean currents are a predominant environmental factor influencing contemporary levels of gene flow between populations, especially in species with pelagic eggs or larvae, or in species with highly migratory adults (Magoulas *et al.*, 2006; Tzeng, 2007; Zhan *et al.*, 2010). The presence of the Benguela Current and the Agulhas Current, may be a type of geographical barrier that influences swordfish distribution patterns directly (*e.g.* the Agulhas Current and Agulhas Current Retroflexion directing the swimming behaviour) or indirectly (*e.g.* influencing the availability of food and creating suitable water temperatures that will attract swordfish). The Agulhas Current is part of the subtropical Indian Ocean gyre (STIOG) and transports about 70–78 Sv ( $1 \text{ Sv} = 10^6 \text{ m}^3 \text{ s}^{-1}$ ) of tropical and subtropical waters along the eastern margin of southern Africa (Lutjeharms, 2007). At the southern tip of Africa, between 16°E and 20°E, the current retroflects with the majority of its waters flowing back into the Indian Ocean as the Agulhas Return Current (Feron *et al.*, 1992).

Only a relative small proportion of the Agulhas Current's warm and salty waters, approximately 2–15 Sv, are transported into the South Atlantic through the Indian-Atlantic Ocean Gateway *via* the Agulhas leakage (de Ruijter *et al.*, 1999; Richardson, 2007). Backeberg *et al.* (2012) suggested that intensified Indian Ocean winds cause enhanced mesoscale variability of the Agulhas Current system, potentially resulting in an increase in Agulhas leakage. Simon *et al.* (2013) concluded that variability in the upstream Agulhas Current hydrography is strongly linked to the dynamics of the Agulhas Return Current and strength of the Southwest Indian Ocean subtropical gyre (SWIOSG) and that

downstream variability in the leakage area (Atlantic sector) at least partly reflects regional variations of the Agulhas Current as a whole.

Surface speeds of the Agulhas Current (AC) may be in excess of 2 m/s (Lutjeharms, 2007). Donohue *et al.* (2000) showed that on occasion the current extends to the bottom whereas on other occasions its vertical penetration was only to a depth of 2300 m. Despite the intense jet-like features of the Agulhas Current, the swordfish have been able to traverse this current from the Atlantic into the Indian Ocean.

In a study by West *et al.* (2012), the horizontal movement patterns of swordfish tagged in 2011 supports the notion that movement is independent of the current and demonstrate that the apparent boundary between the Atlantic and Indian oceans is not insurmountable by this species. The study observed one particular individual that was tagged at 18°E and crossed the 20°E longitude twice, returning to the Atlantic Ocean after swimming into the Indian Ocean as far as 33°E.

Muths *et al.* (2013), with samples of swordfish collected in 2009, concluded that based on the results of mtDNA and microsatellite markers, “the boundary between Atlantic and Indian swordfish populations was not so strict and might be more considered as a transition zone between 17°E and 23°E east that is spatio-temporally driven by the Agulhas Current activity”. Furthermore, their study concluded that the variability observed in the mitochondrial signature of South African samples could be attributed to specific pelagic habitats respectively used by Indian and Atlantic swordfish around South Africa, rather than an ontogenetic migration of individuals from the Atlantic Ocean to the Indian Ocean (Muths *et al.*, 2013).

The results of this study and of Muths *et al.* (2013) indicate admixture or a transition zone in the south in two time periods that are four years apart. The Agulhas Current fluctuates over various time scales (Simon *et al.*, 2013) and regardless of this fluctuation, the results indicate that swordfish will distribute in both directions and is not limited by the strong Agulhas Current. The degree of swordfish intermingling is independent of the variation in the Agulhas Return Current and the Agulhas Current Retroflection.

Evidence of intermingling, gene flow and migration of bigeye tuna around South Africa indicated that a large pelagic stock can surpass this oceanic boundary separating the Atlantic Ocean and Indo-Pacific stocks (Chow *et al.*, 2000). Muths *et al.* (2013) included further examples of dispersal from

the Atlantic Ocean into the Indian Ocean by the hammerhead shark (*Sphyrna lewini*) (Duncan *et al.*, 2006), the green turtle *Chelonia mydas* (Bourjea *et al.*, 2007) and the leatherback turtle *Dermochelys coriacea* (Luschi *et al.*, 2006), all active swimmers.

Kai and Marsac (2010) explored the influence of mesoscale eddies' dynamics in the Mozambique Channel on top predators such as seabirds and tunas. The highest species diversity among longline catches (yellowfin tuna, bigeye tuna, swordfish and albacore tuna) relative to the whole Indian Ocean was recorded in the South Mozambique Channel (Fonteneau, 1997). The study could not exclude that such peculiarities might be related to mesoscale activity that is particularly well-developed in the Mozambique Channel area. Good foraging conditions are promoted along the edge of eddies as a result of the interplay of the maturation process from cyclonic eddies and the concentration process by eddy interactions (Kai and Marsac, 2010). As a result, concentrations of swordfish in the SWIO region could be attributed to favourable feeding opportunities, and these conditions may attract Atlantic Ocean stocks into this region.

The geographical distribution of swordfish in temperate and tropical regions between 60°N and 45°S, and the wide temperature tolerance of between 5°C and 27°C, are characteristics that support inter-ocean distribution (Nakamaru, 1985; Govender *et al.*, 2003). As solitary opportunistic feeders, swordfish are predisposed to swimming where conditions will maximise feeding and growth opportunities. The sex ratio of swordfish catches made by South Africa's pelagic longline fleet is dominated by females, indicating a sex-biased dispersal pattern of females that swim to the southern limits of the species range around South Africa (West *et al.*, unpublished). This has implications for sustainable exploitation of the species in the region.

Evidence of mixed stocks of swordfish has been alluded to by Viñas *et al.* (2007) in the North Atlantic-Mediterranean Sea region, concluding that the Mediterranean stock extends beyond the Strait of Gibraltar to at least 10°W and mixes with the North Atlantic stock. The possibility that the Atlantic-Mediterranean Sea transition is a phylogeographic break (Patarenello *et al.*, 2007) does not beg consideration in the Indian-Atlantic Ocean region. Similarly, the North Atlantic-South Atlantic stocks are two oceanic gyres separated only by the equator. Chow *et al.* (2002) concluded a boundary zone between the two stocks at around 10°N to 2°N and Chow *et al.* (2007) conducted further studies confirming that the boundary between the North Atlantic and South Atlantic at 5°N should be reconsidered. More recently, Smith *et al.* (2015) sampled the North Atlantic, South Atlantic and Mediterranean Sea extensively to study genetic differentiation of 774 individuals,

indicating admixture zones in the North Atlantic and suggesting too that the 5°N management boundary extend to 20°N-25°N from 45°W. It seems that swordfish stock structure between oceans is more complex than the management boundaries indicate.

The possibility cannot be excluded that the weak differentiation observed in the current study between swordfish from the two managerial populations may also reflect their very recent divergence along with presumably large effective population sizes (Benestan *et al.*, 2015). The amount of time since divergence may be insufficient for a significant genetic structure to be revealed by microsatellites. Significant levels of differentiation at neutral loci may be realized only at the largest geographical scales (O'Reilly *et al.*, 2004).

Despite the poor quality of the degraded DNA samples, with resultant smaller sampling size, the results of this study provided evidence for admixture of swordfish between the boundary of the Indian and Atlantic oceans. There is evidence of gene flow and migration in this area in both directions, though the evidence for weak differentiation suggests that the Indian Ocean and Atlantic Ocean contain separate stocks and that swordfish stocks coexist around South Africa but return to their ocean of origin to reproduce. The stock structure at a regional scale in the area around South Africa has been speculated for a long time. Stock assessment workshops have expressed reluctance to include the catch data from South Africa due to the uncertainty of the origin of the reported stock. Although the results of this study indicate weak differentiation, it provides preliminary rather than conclusive evidence of admixed stocks around South Africa between, but not exclusive to, 14°E to 27°E. It is, however, sufficient to motivate for the two tuna Regional Fisheries Management Organisations (trFMOs), ICCAT and IOTC, to begin to reconsider how this stock is managed between the two oceans.

Further investigations into temporal and sex-biased genetic differentiation are needed, including the ecological determinants that drive this differentiation (*e.g.* sea temperature, currents, salinity, food availability) (Banks *et al.*, 2007; Selkoe *et al.*, 2010; White *et al.*, 2010; Benestan *et al.*, 2015). The combined use of nuclear and mitochondrial markers and more comprehensive sampling across this inter-oceanic boundary integrated with local ecological investigations could help to shed more light on the exact ecological process in shaping genetic population structure of swordfish (Benestan *et al.*, 2015). Utilising large numbers of SNP markers could improve regional population structure delineation and population assignment success in a context of weak genetic structure (Benestan *et al.*, 2015), and this marker could therefore also be considered in future studies in this region.

Furthermore, utilising markers that can calculate mixing ratios, such as mtDNA segments in Chow *et al.* (2000), will have practical applications in calculating fishing mortality in the two stocks.

## References

- Adkinson, M. D. (1996). Population differentiation in Pacific salmon: local adaptation, genetic drift, or the environment? *Canadian Journal of Fisheries and Aquatic Sciences*, 52, pp. 2762 – 2777.
- Aguila, R. D., Perez, S. K. L., Catacutan, B. J. N., Lopez, G. V., Barut, N. C. and Santos, M. D. (2015) Distinct yellowfin tuna (*Thunnus albacares*) stocks detected in Western and Central Pacific Ocean (WCPO) using DNA microsatellites. *PLoS ONE*, 10(9), e0138292.
- Albaina A., Iriondo M., Velado I., Laconcha U., Zarraonaindia I., Arrizabalaga H., Pardo M. A., Lutcavage, M., Grant, W.S. and Estonba, A. (2013). Single nucleotide polymorphism discovery in albacore and Atlantic bluefin tuna provides insights into worldwide population structure. *Animal Genetics*, 44, pp. 678 – 692.
- Alvarado Bremer, J. R., Baker, A. J. and Mejuto, J. (1995). Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) populations in the Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Sciences*, 52, pp. 1720 – 1732.
- Alvarado Bremer, J. R., Mejuto, J., Greig, T. W. and Ely, B. (1996). Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. *Journal of Experimental Marine Biology and Ecology*, 197, pp. 295 – 310.
- Alvarado Bremer, J. R., Stequert, B., Robertson, N. W. and Ely, B. (1998). Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus*) populations. *Marine Biology*, 132, pp. 547 - 557.
- Alvarado Bremer, J. R., Mejuto, J., Gómez-Márquez, J., Viñas, J. and Boán, F. (1999). Genetic analysis of bigeye tuna population subdivision. *Collective Volume of Scientific Papers ICCAT*, 49, pp. 370 - 373.
- Alvarado Bremer, J. R., Mejuto, J., Gómez-Márquez, J., Boán, F., Carpintero, P., Rodríguez, J. M., Viñas, J., Greig, T. W. and Ely, B. (2005). Hierarchical analyses of genetic variation of samples from breeding and feeding grounds confirm the genetic partitioning of Northwest Atlantic and South Atlantic populations of swordfish (*Xiphias gladius* L.). *Journal of Experimental Marine Biology and Ecology*, 327, pp. 167 – 182.
- Alvarado Bremer, J. R., Hinton, M. G. and Greig, T. W. (2006). Evidence of spatial genetic heterogeneity in Pacific swordfish (*Xiphias gladius*) revealed by the analysis of LDH-A sequences. *Bulletin of Marine Science*, 79, pp. 493 – 503.

- Alvarado Bremer, J., Mejuto, J., Gómez-Márquez, J., Pla-Zanuy, C., Viñas, J., Marques, C., Hazín, F., Griffiths, M., Ely, B., García-Cortés, B. and Greig, T. W. (2007). Genetic population structure of Atlantic swordfish: current status and future directions. *Collective Volume of Scientific Papers ICCAT*, 61, pp. 107 – 118.
- Amorim, A. F. and Arfelli, C. A. (1980). Reproduccion del pez espada (*Xiphias gladius* L. 1758) en el sudeste y sur del brasil. *Collective Volume of Scientific Papers ICCAT*, 9, pp. 624 - 626.
- Amos, W., Hoffman, J. I., Frodsham, A., Zhang, L., Best, S. and Hill, A.V.S. (2007). Automated binning of microsatellite alleles: problems and solutions. *Molecular Ecology Notes*, 7, pp. 10 – 14.
- Anderson, J. D. and Karel, W. J. (2014) Limited genetic structure of Gulf Menhaden (*Brevoortia patronus*), as revealed by microsatellite markers developed for the genus *Brevoortia* (Clupeidae). *Fishery Bulletin*, 112, pp. 71 - 81.
- Antão, T., Lopes, A., Lopes, R., Beja-Pereira, A. and Luikart, G. (2008). LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, 9: pp. 323.
- Appleyard, S. A., Grewe, P. M., Innes, B. H. and Ward, R. D. (2001). Population structure of yellowfin tuna (*Thunnus albacares*) in the western Pacific Ocean, inferred from microsatellite loci. *Marine Biology*, 139, pp. 383 – 393.
- Appleyard, S. A., Ward, R. D. and Grewe, P. M. (2002). Genetic stock structure of bigeye tuna in the Indian Ocean using mitochondrial DNA and microsatellites. *Journal of fish Biology*, 60, pp. 767 – 770.
- Árnason, E., Pálsson, S. and Arason, A. (1992). Gene flow and lack of population differentiation in Atlantic cod, *Gadus morhua* L., from Island, and comparison of cod from Norway and Newfoundland. *Journal of Fish Biology*, 40, pp. 751 – 770.
- Arocha, F. and Lee, D. W. (1996). Maturity at size, reproductive seasonality, spawning frequency, fecundity and sex ratio in swordfish from the Northwest Atlantic. *Collective Volume of Scientific Papers ICCAT*, 45, pp. 350 - 357.
- Avice, J. C. (2000). *Phylogeography: The History and Formation of Species*. Cambridge, MA: Harvard University Press, pp. 447.
- Backeberg, B. C., Penven, P. and Rouault, M. (2012). Impact of intensified Indian Ocean winds on mesoscale variability in the Agulhas system. *Nature Climate Change*, 2, pp. 608 – 612.
- Banks, M. A., Eichert, W. and Olsen, J. B. (2003). Which genetic loci have greater population assignment power? *Bioinformatics*, 19, pp. 1436 – 1438.
- Banks, S. C., Piggott, M. P., Williamson, J. E., Bové, U., Holbrook, N. J. and Beheregaray, L. B. (2007). Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin. *Ecology*, 88(12), pp. 3055–3064.

- Barker, A. M., Nosal, A. P., Lewallen, E. A. and Burton, R. S. (2015). Genetic structure of leopard shark (*Triakis semifasciata*) populations along the Pacific coast of North America. *Journal of Experimental Marine Biology and Ecology*, 472, pp. 151 – 157.
- Baverstock, P. R. and Moritz, C. (1996). Project design. In: Hillis, D. M., Moritz, C. and Mable, B. K., ed., *Molecular systematics*. 2<sup>nd</sup> ed. Cambridge: Sinauer Associates, pp. 17 – 27.
- Beacham, T. D., Pollard, S. and Le, K. D. (1999). Population structure and stock identification of steelhead in southern British Columbia, Washington, and the Columbia River based on microsatellite DNA variation. *Transactions of the American Fisheries Society*, 128(6), pp. 1068 – 1084.
- Beacham, T. D., Bratney, J., Millar, K. M., Withler, R. E. (2002). Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. *ICES Journal of Marine Science*, 54, pp. 650 – 655.
- Begg, G. A. and Waldman, J. R. (1999). An holistic approach to fish stock identification. *Fisheries Research*, 43, pp. 35-44.
- Belkhir, K., Borsa, P., Chikhi, L., Raufaste N. and Bonhomme, F. (2004). GENETIX 4.02, logiciel sous Windows TM pour la génétique des populations, Laboratoire Génome, Populations, Interactions; CNRS UMR 5000; Université Montpellier II, Montpellier (France).
- Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R. and Bernatchez, L. (2015). RAD-genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species. *Molecular Ecology*, 24, pp. 3299 – 3315.
- Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. and Sayers, E.W. (2009). GenBank. *Nucleic Acids Research*, 37, D26 – 31.
- Bentzen, P., Taggart, C. T., Ruzzante, D. E. and Cook, D. (1996). Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, pp. 2706 – 2721.
- Bernard, A. M, Shivji, M. S., Prince, E. D., Hazin, F. H. V., Arocha, F., Domingo, A. and Feldheim, K. A. (2014). Comparative population genetics and evolutionary history of two commonly misidentified billfishes of management and conservation concern. *BMC Genetics*, 15, pp. 141.
- Billington, N. (2003). Mitochondrial DNA. In: Hallerman, E. M., ed., *Population Genetics- Principles and Applications for Fisheries Scientists*. Bethesda, MD: American Fisheries Society, pp. 59 – 100.
- Borsa, P., Blanquer, A. and Berrebi, P. (1997.) Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. *Marine Biology*, 129, pp. 233 – 246.

- Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *The American Journal of Human Genetics*, 32, pp. 314 – 331.
- Bourjea, J., Lapègue, S., Gagnevin, L., Broderick, D., Mortimer, J.A., Ciccione, S., Roos, D., Taquet, C. and Grizel, H. (2007). Phylogeography of the green turtle, *Chelonia mydas* in the Southwest Indian Ocean. *Molecular Ecology*, 16, pp. 175 – 186.
- Boustany, A. M., Reeb, C. A. and Block, B. A. (2008). Mitochondrial DNA and electronic tracking reveal population structure of Atlantic bluefin tuna (*Thunnus thynnus*). *Marine Biology*, 156, pp. 13 – 24.
- Bradman, H. M., Grewe, P. M. and Appleton, B. (2011). Direct comparison of mitochondrial markers for the analysis of swordfish stock structure. *Fisheries Research*, 109, pp. 95 – 99.
- Brookfield, J. F. Y. (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology*, 5, pp. 4534 – 4555.
- Carlsson, J., McDowell, J. R., Díaz-Jaimes, P., Carlsson, J. E. L., Boles, S.B., Gold, J. R. and Graves, J. E. (2004). Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea. *Molecular Ecology*, 13, pp. 3345 – 3356.
- Carvalho, G. R., and Hauser, L. (1994). Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries*, 4, pp. 326 – 350.
- Castro, J.A., Picornell, A. and Ramon, M. (1998). Mitochondrial DNA: a tool for populational genetics studies. *International Microbiology*, 1, pp. 327 – 332.
- Chakraborty, R., De Andrade, M., Daiger, S. P. and Budowle, B. (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics*, 56, pp. 45 – 57.
- Chen, C., Durand, E., Forbes, F. and François, O. (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, 7, pp. 747 – 756.
- Cheng, J., Yanagimoto, T., Song, N. and Gao, T. (2015). Population genetic structure of chub mackerel *Scomber japonicas* in the Northwestern Pacific inferred from microsatellite analysis. *Molecular Biology Reports*, 42, pp. 373 – 382.
- Chiang, H. C., Hsu, C. C., Lin, H. D., Ma, G. C., Chiang, T. Y. and His, Y. Y. (2006). Population structure of bigeye tuna (*Thunnus obesus*) in the South China Sea, Philippine Sea and Western Pacific Ocean inferred from mitochondrial DNA. *Fisheries Research*, 79, pp. 219 – 225.



- Chiang, H. C., Hsu, C. C., Wu, G. C. C., Chang, S. K. and Yang, H. Y. (2008). Population structure of bigeye tuna (*Thunnus obesus*) in the Indian Ocean inferred from mitochondrial DNA. *Fisheries Research*, 90, pp. 305 – 312.
- Chow, S., Okamoto, H., Uozumi, Y., Takeuchi, Y. and Takeyama, H. (1997). Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. *Marine Biology*, 127, pp. 359 – 367.
- Chow, S., Okamoto, H., Miyabe, N., Hiramatsu, K. and Barut, N. (2000). Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology*, 9, pp. 221 – 227.
- Chow, S. and Takeyama, H., (2000). Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. *Journal of Fish Biology*, 56(5), pp. 1087 – 1098.
- Chow, S., Nohara, K., and Takeuchi, Y. (2002). Boundary between North and South Atlantic stocks of swordfish (*Xiphias gladius*): an implication from nuclear DNA analysis. *Collective Volume of Scientific Papers ICCAT*, 54, pp. 1544 – 1546.
- Chow, S., Clarke, S., Nakadate, M., and Okazaki, M. (2007). Boundary between the North and South Atlantic populations of the swordfish (*Xiphias gladius*) inferred by a single nucleotide polymorphism at calmodulin gene intron. *Marine Biology*, 152, pp. 87 - 93.
- Çiftçi, Y. and Okumuş, I. (2002). Fish population genetics and applications of molecular markers to fisheries and aquaculture: I- Basic Principles of Fish Population Genetics. *Turkish Journal of Fisheries and Aquatic Sciences*, 2, pp. 145 – 155.
- Clark, T. B., Ma, L., Saillant, E. and Gold, J. R. (2004). Microsatellite DNA markers for population-genetic studies of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) and other species of genus *Thunnus*. *Molecular Ecology Notes*, 4, pp. 70 – 73.
- Coates, A. G., Jackson, J. B. C., Collins, L. S., Cronin, T. M., Dowsett, H. J., Bybell, L. M., Jung, P. and Obando, J. A. (1992). Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. *Geological Society of America Bulletin*, 104, pp. 814 – 828.
- Cornuet, J. M., Piry, S., Luikart, G., Estoup, A. and Solignac, M. (1999). New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, 153, pp. 1989 – 2000.
- Dammannagoda, S. T., Hurwood, D. A., and Mather, P. B. (2008). Evidence for fine geographical scale heterogeneity in gene frequencies in yellowfin tuna (*Thunnus albacares*) from the north Indian Ocean around Sri Lanka. *Fisheries Research*, 90, pp. 147 – 157.

- Dammannagoda, S. T., Hurwood, D. A. and Mather, P. B. (2011). Genetic analysis reveals two stocks of skipjack tuna (*Katsuwonus pelamis*) in the north western Indian Ocean. *Canadian Journal of Fisheries and Aquatic Sciences*, 68(2), pp. 210 – 223.
- Davies, C. A., Gosling, E. M., Was, A., Brophy, D. and Tysklind, N. (2011). Microsatellite analysis of albacore tuna (*Thunnus alalunga*): population genetic structure in the North-East Atlantic Ocean and Mediterranean Sea. *Marine Biology*, 158, pp. 2727 – 2740.
- De Innocentiis, S., Sola, L., Cataudella, S. and Bentzen, P. (2001). Allozyme and microsatellite loci provide discordant estimates of population differentiation in the endangered dusky grouper (*Epinephelus marginatus*) within the Mediterranean Sea. *Molecular Ecology*, 10, pp. 2163 – 2175.
- de Ruijter, W. P. M., Biastoch, A., Drijfhout, S. S., Lutjeharms, J. R. E., Matano, R. P., Pichevin, T., van Leeuwen, P. J., and Weijer, W. (1999). Indian-atlantic interocean exchange: Dynamics, estimation and impact. *Journal of Geophysical Research*, 104, pp. 20 885–20 910.
- De Woody, J. A. and Avise, J. C. (2000). Microsatellite variation in marine, freshwater, and anadromous fishes compared with other animals. *Journal of Fish Biology*, 56, pp. 461 – 473.
- Diaz-Jaimes, P., Uribe-Alcocer, M., Ortega-García, S. and Durand, J.D. (2006). Spatial and temporal mitochondrial DNA genetic homogeneity in dolphinfish populations (*Coryphaena hippurus*) in the eastern central Pacific. *Fisheries Research*, 80, pp. 333 – 338.
- Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J. and Ovenden, J. R. (2014). NEESTIMATOR v2: re-implementation of software for the estimation of contemporary effective population size ( $N_E$ ) from genetic data. *Molecular Ecology Resources*, 14, pp. 209 – 214.
- Donohue, K. A., Firing, E. and Beal, L. (2000). Comparison of three velocity sections of the Agulhas Current and Agulhas Undercurrent. *Journal of Geophysical Research*, 105(12), pp. 28 585 – 28 593.
- Durand, J. D., Collet, A., Chow, S., Guinand, B. and Borsa, P. (2005). Nuclear and mitochondrial DNA markers indicated unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa. *Marine Biology*, 147, pp. 313 – 322.
- Durand, E., Jay, F., Gaggiotti, O. E. and François, O. (2009). Spatial inference of admixture proportions and secondary contact zones, *Molecular Biology and Evolution*, 26, 1963 – 1973.
- Duncan, K. M., Martin, A. P., Bowen, B. W. and De Couet, H. G. (2006). Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). *Molecular Ecology*, 15, pp. 2239 – 2251.
- Earl, D. A. and von Holdt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*. 4, pp. 359 – 361.

- Edwards, A. W. F. (2008). G. H. Hardy (1908) and Hardy-Weinberg Equilibrium. *Genetics*, 179, pp. 1743 – 1150.
- Ellegren, H. (2004). *Microsatellites: simple sequences with complex evolution*. *Nature Reviews Genetics*, 5, pp. 435 – 445.
- Elliott, N. G. and Ward, R. D. (1992). Enzyme variation in orange roughy (*Hoplostethus atlanticus*) samples from southern Australian and New Zealand waters. *Australian Journal of Marine and Freshwater Research*, 45, pp. 51 – 67.
- Ely, B., Viñas, J., Alvarado Bremer, J., Jaime, R., Black, D., Lucas, L., Covello, K., Labrie, A. and Thelen, E. (2005). Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). *BMC Evolutionary Biology*, 5, p. 19.
- Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14, pp. 2611 – 2620.
- Excoffier, L., Smouse, P. E., Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131, pp. 479–491.
- Excoffier, L. and Lischer, H. E. L. (2010). Arlequin suite ver 3.5.2.1: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, pp. 564 – 567.
- Fabbri, E., Caniglia, R., Mucci, N., Thomsen, H. P., Krag, K., Pertoldi, C., Loeschcke, V. and Randi, E. (2012). Comparison of single nucleotide polymorphisms and microsatellites in non-invasive genetic monitoring of a wolf population. *Archives of Biological Science Belgrade*, 64, pp. 321 – 335.
- Falush, D., Stephens, M. and Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164, pp. 1567 – 1587.
- Farnham, T. T. (2003). Genetic variation in Atlantic yellowfin tuna (*Thunnus albacares*) to assess stock structure and reproductive variance. Unpublished Master of Science Thesis. Texas A&M University.
- Feldheim, K. A., Gruber, S. H. and Ashley, M. V. (2001). Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Molecular Ecology*, 10, pp. 295 – 303.
- Feron, R. C. V., De Ruijter, P. M. and Oskam, D. (1992). Ring shedding in the Agulhas Current System. *Journal of Geophysical Research*, 97(C6), pp. 9 467 – 9 477.

- Garcia, A., Cecchetti, S., Santos, M. N., Mattiucci, S., Nascetti, G. and Cimmaruta, R. (2011). Population structure of Atlantic Swordfish (*Xiphias gladius* L. 1758) (Teleostea, Xiphiidae) using mitochondrial DNA analysis: implications for fisheries management. *Animal Biodiversity and Conservation*, 34, pp. 133 – 140.
- García-Cortés, B., Mejuto, J. and Quintans, M. (2003). Summary of swordfish (*Xiphias gladius*) recaptures carried out by the Spanish surface longline fleet in the Atlantic Ocean: 1984-2002. *Collective Volume of Scientific Papers ICCAT*, 55(4), pp. 1476 – 1484.
- Gillespie, J. H. (1998). Population Genetics: A Concise Guide. Baltimore: The Johns Hopkins University Press.
- Gjøsaeter, J., Jørstad, K. E., Nævdal, G. and Thorkildsen, S. (1992). Genotype distributions of cod from the Norwegian Skagerrak coast. *Sarsia*, 76, pp. 225 – 259.
- Gold, J. R., King, T. L., Richardson, L. R., Bohlmeier, D. H. and Matlock, G. C. (1994). Genetic studies in marine fishes. VII: allozyme differentiation within and between red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. *Journal of Fish Biology*, 116, pp. 517 – 185.
- González, E.G., Beerli, P. and Zardoya, R. (2008). Genetic structuring and migration patterns of Atlantic bigeye tuna, *Thunnus obesus* (Lowe, 1839). *BMC Evolutionary Biology*, 8, pp. 252.
- Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available at: <http://www2.unil.ch/popgen/softwares/fstat.htm> [Accessed 1<sup>st</sup> February 2016].
- Govender, A., van der Elst, R. and James, N. (2003). Swordfish. Global lessons. 1<sup>st</sup> ed. [pdf] South Africa: WWF-South Africa. Available at: <http://hdl.handle.net/1834/921> [Accessed 1 February 2016].
- Graves, J. E. and McDowell, J. R. (2001). A genetic perspective on the stock structures of blue marlin and white marlin in the Atlantic Ocean. *Collective Volume of Scientific Papers ICCAT*, 53, pp. 180 – 187.
- Graves, J. E. and McDowell, J. R. (2003). Stock structure of the worlds Istiophorid billfishes; a genetic perspective. *Marine and Freshwater Research*, 54, pp. 287 – 298.
- Graves, J. E. and McDowell, J. R. (2006). Genetic analysis of white marlin (*Tetrapturus albidus*) stock structure. *Bulletin of Marine Science*, 79, pp. 469 – 482.
- Greig, T. W., Alvarado Bremer, J. R. and Ely, B. (1999). Preliminary results from genetic analyses of nuclear markers in swordfish, *Xiphias gladius*, reveal concordance with mitochondrial DNA analysis. *Collective Volume of Scientific Papers ICCAT*, 49, pp. 476 – 482.

- Grewe, P. (1997). An assessment of bigeye (*Thunnus obesus*) population structure in the Pacific Ocean, based on Mitochondrial DNA and DNA microsatellites analysis. Working paper for the 7<sup>th</sup> Meeting of the Western Pacific Yellowfin Tuna Research Group, Nadi, Fiji, June 18 – 20.
- Grijalva-Chon, J. M., Numachi, K., Sosa-Nishizaki, O. and de la Rosa-Velez, J. (1994). Mitochondrial DNA analysis of North Pacific swordfish *Xiphias gladius* population structure. *Marine Ecology Progress Series*, 115, pp. 15 – 19.
- Hartl, D.L. and Clark, A.G. (1997). Principles of Population Genetics, 3<sup>rd</sup> ed. Sinauer Associates, Inc, Sunderland, MA.
- Hellberg, M. E. (2009). Gene flow and isolation among populations of marine animals. *Annual Review of Ecology Evolution and Systematics*, 40, pp 291 – 310.
- Herráeza, D. L., Schäfer, H., Mosner, J., Fries, H. R. and Wink, M. (2005). Comparison of microsatellite and single nucleotide polymorphism markers for the genetic analysis of a Galloway cattle population. *Zeitschrift für Naturforschung C*, 60, pp. 637 – 643.
- Hinton, M. G., and Alvarado Bremer, J. (2007). Stock structure of swordfish in the Pacific Ocean. IATTC Working Group to Review Stock Assessments, 8th Meeting, La Jolla, CA, 7 – 11 May 2007. *Document SAR-8-11*.
- Holdsworth, J. C., Sippel, T. J., and Paul, P. J. (2007). An investigation into swordfish stock structure using satellite tag and release methods. Biology Specialist Working Group Paper *WCPFC-SC3-2007/BI WP-3* [Accessed 15 February 2016].
- Hubisz, M.J., Falush, D., Stephens, M. and Pritchard, J.K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9, pp. 1322 – 1332.
- ICCAT (2013). Report of the Standing Committee on Research and Statistics (SCRS). [online] Madrid, Spain: ICCAT. Available at: <http://www.iccat.es/en/meetings.asp> [Accessed 1 February 2016].
- IOTC (2013). Report of the Sixteenth Session of the IOTC Scientific Committee. [online] Busan, Republic of Korea: IOTC. Available at: <http://www.iotc.org/meetings/16th-session-scientific-committee> [Accessed 1 February 2016].
- Jakobsson, M. and Rosenberg, N. A. (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23, pp. 1801 – 1806.
- Jarne, P. and Lagoda, P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, 11, pp. 424 – 429.

- Jean, C., Bourjea, J., Jouen, E. and Taquet, M. (2006). Stock structure of the swordfish (*Xiphias gladius*) in the Southwest Indian Ocean: a preliminary study. *Bulletin of Marine Science*, 79, pp. 521 – 526.
- Kai, E. T. and Marsac, F. (2010). Influence of mesoscale eddies on spatial structuring of top predators' communities in the Mozambique Channel. *Progress in Oceanography*, 86, pp. 214 – 223.
- Kalinowski, S. T., Taper, M. L. and Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16, pp. 1099 – 1106.
- Karlsson, S. and Mork, J. (2005). Deviations from Hardy –Weinberg equilibrium, and temporal instability in allele frequencies at microsatellite loci in a local population of Atlantic cod. *ICES Journal of Marine Science*, 62, pp. 1588 –1596.
- Kasapidis, P., Mejuto, J., Tserpes, G., Antoniou, A., García-Cortés, B., Peristeraki, P., Oikonomaki, K., Kotoulas, G. and Magoulas, A. (2006). Genetic structure of the swordfish (*Xiphias gladius*) stocks in the Atlantic using microsatellite DNA analysis. *Collective Volume of Scientific Papers ICCAT*, 61, pp. 89 – 98.
- Kasapidis, P., Magoulas, A., García-Cortés, B., and Mejuto, J. (2008). Stock structure of swordfish (*Xiphias gladius*) in the Pacific Ocean using microsatellite DNA markers. Biology Specialist Working Group Paper *WCPFC-SC4-2008/BI WP-4*. [Accessed 15 February 2016].
- Kasapidis, P., Pakaki, V., Kotoulas, G. and Magoulas, A. (2009). Isolation and characterization of 18 new polymorphic microsatellite loci for the swordfish, *Xiphias gladius*. *Molecular Ecology Resources*. 9, pp. 1383 – 1386.
- Keigwin, L. D. (1982). Isotopic paleoceanography of the Caribbean and east Pacific: Role of Panama uplift in Late Neogene time. *Science*, 217, pp. 350 – 352.
- Knutsen, H., Jorde, P. E., Andre, C. and Stenseth, C. H. R. (2003). Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. *Molecular Ecology*, 12, pp. 385 – 394.
- Kotoulas, G., Mejuto, J., Tserpes, G., García-Cortés, B., Peristeraki, P., De la Serna, J. M. and Magoulas, A. (2003). DNA microsatellite markers in service of swordfish stock-structure analysis in the Atlantic and Mediterranean. *Collective Volume of Scientific Papers ICCAT*, 55, 1632 – 1639.
- Kotoulas, G., Mejuto, J., Antoniou, A., Kasapidis, P., Tserpes, G., Piccinetti, C., Peristeraki, P., García-Cortés, B., Oikonomaki, K., De la Serna, J. M. and Magoulas, A. (2006). Global genetic structure of swordfish (*Xiphias gladius*) as revealed by microsatellite DNA markers. *Collective Volume of Scientific Papers ICCAT*, 61, 79 – 88.

- Kunal, S. P., Kumar, G., Menezes, M. R. and Meena, R. M. (2013). Mitochondrial DNA analysis reveals three stocks of yellowfin tuna *Thunnus albacares* (Bonnaterre, 1788) in Indian waters. *Conservation Genetics*, 14, pp. 205 – 213.
- Laconcha, U., Iriando, M., Arrizabalaga, H., Manzano, C., Markaide, P., Montes I, Zarraonaindia, I., Velado, I., Bilbao, E., Goni, N., Santiago, J., Domingo, A., Karakulak, S., Oray, I. and Estonba, A. (2015). New nuclear SNP markers unravel the genetic structure and effective population size of albacore tuna (*Thunnus alalunga*). *PLoS ONE*, 10: e0128247.
- Latch, E. K., Dharmarajan, G., Glaubitz, J. C. and Rhodes, O. E. (2006). Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, 7, pp. 295 – 302.
- Lessios, H. A. and Weinberg, J. R. (1993). Migration, gene flow and reproductive isolation between and within morphotypes of the isopod *Excirrolana* in two oceans. *Heredity*, 71, pp. 561 – 573.
- Li, Y., Bence, J. R. and Brenden, T. O. (2015). An evaluation of alternative assessment approaches for intermixing fish populations: a case study with Great Lakes lake whitefish. *ICES Journal of Marine Science*, 72, pp. 70 – 81
- Li, W., Chen, X., Xu, Q., Zhu, J., Dai, X. and Xu, L. (2015) (1). Genetic population structure of *Thunnus albacares* in the central Pacific Ocean based on mtDNA COI gene sequences. *Biochemical Genetics*, 53, pp. 8 – 22.
- Lu, C. P., Chen, C. A., Hui, C. F., Tzeng, T. D. and Yeh, S. Y. (2006). Population genetic structure of the swordfish, *Xiphias gladius* (Linnaeus, 1758), in the Indian Ocean and West Pacific inferred from the complete DNA sequence of the mitochondrial control region. *Zoological Studies*, 45, pp. 269 – 279.
- Lundy, C. J., Rico, C. and Hewitt, G. (2000). Temporal and spatial genetic variation in spawning grounds of European hake (*Merluccius merluccius*) in the Bay of Biscay. *Molecular Ecology*, 9, pp. 2067 – 2079.
- Luschi, P., Lutjeharms, J. R. E., Lambardia, P., Mencacci, R., Hughes, G. R. and Hays, G. C. (2006). A review of migratory behaviour of sea turtles off Southeastern Africa. *South African Journal of Science*, 102, pp. 51 – 58.
- Magoulas, A., Kotoulas, G., de la Serna, J. M., De Metrio, G., Tsimenides, N. and Zouros, E. (1993). Genetic structure of swordfish (*Xiphias gladius*) populations of the Mediterranean and the eastern side of the Atlantic: analysis by mitochondrial DNA markers. *Collective Volume of Scientific Papers ICCAT*, 40, 126 – 136.
- Magoulas, A., Castillho, R., Caetano, S., Marcato, S. and Patarnello, T. (2006). Mitochondrial DNA reveals a mosaic pattern of phylogeographical structure in Atlantic and Mediterranean

- populations of anchovy (*Engraulis encrasicolus*). *Molecular Phylogenetics and Evolution*, 39, pp. 734 – 746.
- Manel, S., Gaggiotti, O. E. and Waples, R. S. (2005). Assignment methods: Matching biological questions with appropriate techniques. *Trends in Ecology and Evolution*, 20, pp. 136 – 142.
- May, B. and Krueger, C. C. (1990). Use of allozyme data for population analysis. In: Whitmore, D.H., ed., *Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management*. Boston: CRC Press, pp. 157 – 171.
- McDowell, J. R. and Graves, J. E. (2008). Population structure of striped marlin (*Kajikia audax*) in the Pacific Ocean based on analysis of microsatellite and mitochondrial DNA. *Canadian Journal of Fisheries and Aquatic Sciences*, 65, pp. 1307 – 1320.
- McPherson, A. A., O'Reilly, P. T., McParland, T. L., Jones, M. W. and Bentzen, P. (2001). Isolation of nine novel tetranucleotide microsatellites in Atlantic herring (*Clupea harengus*). *Molecular Ecology Notes*, 1, pp. 31 – 32.
- McQuinn, I. H. (1997). Metapopulations and the Atlantic herring. *Reviews in Fish Biology and Fisheries*, 7, pp. 297 – 329.
- Menezes, M. R., Noguchi, D., Nakajima, M. and Taniguchi, M. (2008). Microsatellite development and survey of genetic variation in skipjack tuna *Katsuwonus pelamis*. *Journal of Fish Biology*, 73, pp. 463 – 473.
- Montes, I., Iriondo, M., Manzano, C., Arrizabalaga, H., Jiménez, E., Pardo, M. A., Goñi, N., Davies, C. A. and Estonba, A. (2012). Worldwide genetic structure of albacore (*Thunnus alalunga*) revealed by microsatellite DNA markers. *Marine Ecology Progress Series*, 471, pp. 183 – 191.
- Morin, P. A., Archer, F. I., Pease, V. L., Hancock-Hanser, B. L., Robertson, K. M., Huebinger, R. M., Martien, K. K., Bickham, J. W., George, J. C., Postma, L. D. and Taylor, B. L. (2012). Empirical comparison of single nucleotide polymorphisms and microsatellites for population and demographic analyses of bowhead whales. *Endangered Species Research*, 19, pp. 129 – 147.
- Mork, J., Ryman, N., Ståhl, G., Utter, F., and Sundnes, G. (1985). Genetic variation in Atlantic cod (*Gadus morhua*) throughout its range. *Canadian Journal of Fisheries and Aquatic Sciences*, 42, pp. 1580 – 1587.
- Muths, D., Grewe, P., Jean, C. and Bourjea, J. (2009). Genetic population structure of the Swordfish (*Xiphias gladius*) in the southwest Indian Ocean: sex-biased differentiation, congruency between markers and its incidence in a way of stock assessment. *Fisheries Research*, 97, pp. 263 – 269.
- Muths, D., Le Couls, S., Evano, H., Grewe, P. and Bourjea, J. (2013). Multi-genetic marker approach and spatio-temporal analysis suggest there is a single panmictic population of Swordfish *Xiphias gladius* in the Indian Ocean. *PloS ONE*, 8: e63558.



- Nakadate, M., Viñas, J., Corriero, A., Clarke, S., Suzuki, N. and Chow, S. (2005). Genetic isolation between Atlantic and Mediterranean albacore populations inferred from mitochondrial and nuclear DNA markers. *Journal of Fish Biology*, 66, pp. 1545 – 1557.
- Nakamura, I. (1985). FAO species catalogue. Vol 5. Billfishes of the world. An annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date. FAO fisheries symposium. 5, pp. 65.
- Nei, M. (1972). Genetic distance between populations. *American Naturalist*, 106, pp. 283 – 291.
- Neilson, J. D., Paul, S. D. and Smith, S. C. (2007). Stock structure of swordfish (*Xiphias gladius*) in the Atlantic: a review of the non-genetic evidence. *Collective Volume of Scientific Papers ICCAT*, 61, pp. 25 – 60.
- Neilson, J., Arocha, F., Calay, S., Mejuto, J., Ortiz, M., Scott, G., Smith, C., Travassos, P., Tserpes, G. and Andrushchenko, I. (2013). The recovery of Atlantic swordfish: the comparative roles of the regional fisheries management organization and species biology. *Reviews in Fisheries Science*, 21, pp. 59 – 57.
- Nesbø, C. L., Rueness, E. K., Iversen, S. A., Skagen, D. W. and Jakobsen, K. S. (2000). Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a genealogical approach reveals genetic structuring among the eastern Atlantic stocks. *Proceedings of the Royal Society of London B: Biological*. 267, pp. 281 – 292.
- Nomura, S., Kobayashi, T., Agawa, Y., Margulies, D., Scholey, V., Sawada, Y. and Yagishita, N. (2014) Genetic population structure of the Pacific bluefin tuna *Thunnus orientalis* and the yellowfin tuna *Thunnus albacares* in the North Pacific Ocean. *Fisheries Science*, 80, pp. 1193 – 1204.
- O'Connell, M. and Wright, J. M. (1997). Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries*, 7, pp. 331 – 363.
- O'Leary, S. J., Hice, L. A., Feldheim, K. A., Frisk, M. G., McElroy, A. E., Fast, M. D. and Chapman, D. D. (2013) Severe inbreeding and small effective number of breeders in a formerly abundant marine fish. *PLoS One*, 8, e66126.
- O'Reilly, P. and Wright, J. M. (1995). The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *Journal of Fish. Biology*, 47, pp. 29 – 55.
- O'Reilly, P. T., Canino, M. F., Bailey, K. M. and Bentzen, P. (2004) Inverse relationship between  $f_{st}$  and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Molecular Ecology*, 13, pp. 1799 – 1814.
- Okumuş, I. and Çiftçi, Y. (2003). Fish population genetics and molecular markers: II- Molecular markers and their applications in fisheries and aquaculture. *Turkish Journal of Fisheries and Aquatic Sciences*, 3, pp. 51 – 79.

- Paetkau, D., Slade, R., Burden, M. and Estoup, A. (2004). Direct, real-time estimation of migration rate using assignment methods: a simulation-based exploration of accuracy and power. *Molecular Ecology*, 13, pp. 55 – 65.
- Patarnello, T., Volckaert, F. A. and Castilho, R. (2007). Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break? *Molecular Ecology*, 16, pp. 4426 – 4444.
- Pemberton, J. M., Slate, J., Bancroft, D. R. and Barrett, J. A. (1995). Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, 4, pp. 249 – 252.
- Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. and Estoup, A. (2004). GeneClass2: a software for genetic assignment and first generation migrants detection. *Journal of Heredity*, 95, pp. 536 – 539.
- Pla, C., Pujolar, J. M. and Viñas, J. (1998). Population genetics and stock structure of large pelagic species (*Thunnus thynnus*, *Thunnus alalunga*, *Xiphias gladius* and *Sarda sarda*) in the Mediterranean. *Collective Volume of Scientific Papers ICCAT*, 50, pp. 209 – 212.
- Pogson, G. H., Mesa, K. H. and Boutilier, R. G. (1995). Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. *Genetics*, 139, pp. 375 – 385.
- Pogson, G. H. (2001). Nucleotide polymorphism and natural selection at the pantophysin (Pan I) locus in the Atlantic cod, *Gadus morhua* (L.). *Genetics*, 157, pp. 317 – 330.
- Pritchard, J. K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, pp. 945 – 959.
- Pritchard, J. and Wen, W. (2004). Department of Human Genetics, University of Chicago, 920 E 58th st, CLCS 507, Chicago IL 60637, USA.
- Provine, W. B. (2004). Ernst Mayr: Genetics and speciation. *Genetics*, 167, 1041 – 1046.
- Pujolar, J. M., Roldán, M. I. and Pla, C. (2003). Genetic analysis of tuna populations, *Thunnus thynnus* and *T. alalunga*. *Marine Biology*, 143, pp. 613 – 621.
- Puncher, G. N., Arrizabalaga, H., Alemany F., Cariani A., Oray I. K., Karakulak F. S., Basilone, G., Cuttutta, A., Mazzola, S., and Tinti, F. (2015). Molecular identification of Atlantic bluefin tuna (*Thunnus thynnus*, Scombridae) larvae and development of a DNA character-based identification key for Mediterranean scombrids. *PLoS ONE*, 10, e0130407.
- Purcell, C. M., and Edmands, S. (2011). Resolving the genetic structure of striped marlin, *Kajikia audax*, in the Pacific Ocean through spatial and temporal sampling of adult and immature fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 68, pp. 1861 – 1875.

- Rannala, B. and Mountain, J. L. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences USA*, 94, pp. 9197 – 9201.
- Raymond, M. and Rousset, F. (1995). GENEPOP (version 4.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, pp. 248 – 249.
- Reeb, C. and Block, B. A. (1997). The usefulness of mitochondrial DNA studies to define management units of the swordfish, *Xiphias gladius*: a review of current literature. *Collective Volume of Scientific Papers ICCAT*, 46, pp. 390 – 392.
- Reeb, C. A., Arcangeli, L. and Block, B. A. (2000). Structure and migration corridors in Pacific populations of the swordfish *Xiphias gladius*, as inferred through analyses of mitochondrial DNA. *Marine Biology*, 136, 1123 – 1131.
- Reeb, C., Arcangeli, L., and Block, B. (2003). Development of 11 microsatellite loci for population studies in the swordfish, *Xiphias gladius* (Teleostei: Scombridae). *Molecular Ecology Notes*, 3, pp. 147 – 149.
- Riccioni, G., Stagioni, M., Landi, M., Ferrara, G., Barbujani G, and Tinti, F. (2013). Genetic structure of bluefin tuna in the Mediterranean Sea correlates with environmental variables. *PLoS ONE*, 8, e80105.
- Rice, W. (1989). Analyzing tables of statistical tests. *Evolution*, 43, pp. 223 – 225.
- Richardson, P. L. (2007). Agulhas leakage into the Atlantic estimated with subsurface floats and surface drifters. *Deep Sea Research Part I: Oceanographic Research Papers*, 54(8), pp. 1361 – 1389.
- Rosel, P. E., and Block, B. A. (1996). Mitochondrial control region variability and global population structure in the swordfish *Xiphias gladius*. *Marine Biology*, 125, pp. 11 – 22.
- Rosenberg, N. A. (2007). DISTRUCT: a program for the graphical display of population structure. Rosenberg lab at Stanford University. Available at: <http://www.stanford.edu/group/rosenberglab/distruct.html> [Accessed 1<sup>st</sup> February 2016].
- Rousset, F. (2008). Genepop'007: a complete reimplement of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, 8, pp. 103 – 106.
- Ruzzante, D. E., Taggart, C. T. and Cook, D. (1998). A nuclear DNA basis for shelf- and bank- scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges bank. *Molecular Ecology*, 7, pp. 1633 – 1680.
- Ruzzante, D. E., Taggart, C. T. and Cook, D. (1999). A review of evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. *Fisheries Research*, 43, pp. 79 – 97.

- Saghai-Maroofof, M. A., Soliman, K. M., Jorgensen, R. A. and Allard, R. W. (1984). Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences*, 81, pp. 8014 – 8019.
- Saxton, B. L. (2009). Historical demography and genetic population structure of the blackfin tuna (*Thunnus atlanticus*) from the Northwest Atlantic Ocean and the Gulf of Mexico. Unpublished Master of Science Thesis. Texas A&M University.
- Schneider, S., Roessli, D. and Excoffier, L. (2000). Arlequin: a software for population genetics data analysis. User manual ver 2.000. Ver. 2.000. Geneva: Genetics and Biometry Lab, Department of Anthropology, University of Geneva.
- Schrey, A. W. and Heist, E. J. (2003). Microsatellite analysis of population structure in the shortfin mako (*Isurus oxyrinchus*). *Canadian Journal of Fisheries Aquatic Sciences*, 60, pp. 670 – 675.
- Schrey, A. W. and Heist, E. J. (2007). Stock structure of pallid sturgeon analyzed with microsatellite loci. *Journal of Applied Ichthyology*, 23, pp. 297 – 303.
- Shriver, M. D., Jin, L., Ferrell, R. E. and Deka, R. (1997). Microsatellite data support an early population expansion in Africa. *Genome Research*, 7, pp. 586 – 591.
- Scoles, D. R. and Graves, J. E. (1993). Genetic analysis of the population structure of yellowfin tuna, *Thunnus albacares*, from the Pacific Ocean. *Fishery Bulletin*, 9(4), pp. 690 – 698.
- Selkoe, K. A. and Toonen, R. J. (2006) Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecological Letters*, 9, pp. 615 – 629.
- Selkoe, K. A., Watson, J. R., White, C., Horin, T. B., Iacchei, M., Mitarai, S., Siegel, D. A., Gaines, S. D. and Toonen, R. J. (2010). Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Molecular Ecology*, 19, pp. 3708 – 3726.
- Shaklee, J. B. and Bentzen, P. (1998) Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science*, 62, pp. 589 – 621.
- Sharp, G. D. (1978). Behavioural and physiological properties of tunas and their effects on vulnerability to fishing gear. In: Sharp, G.D. and Dizon, A.E., ed., *The Physiological Ecology of Tunas*. New York: Academic Press, pp. 397 – 449.
- Shaw, P. W., Turan, C., Wright, J. M., O'Connell, M. and Carvalho, G. R. (1999). Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses. *Heredity*, 83, pp. 490 – 499.
- Simon, M. H., Arthus, K. L., Hall, I. R., Peeters, F. J. C., Loveday, B. R., Barker, S., Ziegler, M. and Zahn, R. (2013). Millennial-scale Agulhas current variability and its implications for salt-leakage through the Indian-Atlantic Ocean Gateway. *Earth and Planetary Science Letters*, 383, pp. 101-112.

- Smith, B. L. and Alvarado Bremer, J. R. (2010). Inferring population admixture with multiple nuclear genetic markers and bayesian genetic clustering in Atlantic swordfish (*Xiphias gladius*). *Collective Volume of Scientific Papers ICCAT*, 65, pp. 185 – 190.
- Smith, B. L., Lu, C-P., García-Cortés, B., Viñas, J., Yeh, S-Y. and Alvarado Bremer, J. R. (2015). Multilocus bayesian estimates of intra-oceanic genetic differentiation, connectivity, and admixture in Atlantic swordfish (*Xiphias gladius* L.). *PLoS ONE*, 10: e0127979.
- Sorenson, L., McDowell, J. R., Knot, T. and Graves, J. E. (2013). Assignment test method using hypervariable markers for blue marlin (*Makaira nigricans*) stock identification. *Conservation Genetics Resources*, 5, pp. 293 – 297.
- Takagi, M., Okamura, T., Chow, S. and Taniguchi, N. (2001). Preliminary study of albacore (*Thunnus alalunga*) stock differentiation inferred from microsatellite DNA analysis. *Fishery Bulletin*, 99, pp. 697 – 701.
- Tserpes, G. and Tsimenides, N. (1995). Determination of age and growth of swordfish, *Xiphias gladius* L., 1758, in the eastern Mediterranean using anal-fin spines. *Fishery Bulletin*, 93(3), pp. 594 – 602.
- Tzeng, T. D. (2007). Population structure and historical demography of the Spotted Mackerel (*Scomber australasicus*) off Taiwan inferred from mitochondrial control region sequencing. *Zoological Studies*, 46, pp. 656 – 663.
- Van Oosterhout, C., Hutchinson, W. F., Wills, P. M. D. and Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4, pp. 535 – 538.
- Van Oosterhout, C., Joyce, D. A. and Cummings, S.M. (2006). Evolution of MHC class IIB in the genome of wild and ornamental guppies, *Poecilia reticulata*. *Heredity*, 97, pp. 111 – 118.
- Viñas, J., Pujolar, J. M., Alvarado Bremer, J. Ely, B. and Pla, C. (1998). Population genetics of swordfish (*Xiphias gladius*) in the Mediterranean Sea, based on allozyme electrophoresis and mitochondrial DNA sequencing. *Collective Volume of Scientific Papers ICCAT*, 50, pp. 225 – 233.
- Viñas, J., Alvarado Bremer, J. R. and Pla, C. (2004). Inter-oceanic genetic differentiation among albacore (*Thunnus alalunga*) populations. *Marine Biology*, 145, pp. 225 – 232.
- Viñas, J., Alvarado Bremer, J., Mejuto, J., De la Serna, J. M., García-Cortés, B. and Pla, C. (2007). Swordfish genetic population structure in the North Atlantic and Mediterranean. *Collective Volume of Scientific Papers ICCAT*, 61, pp. 99 – 106.
- Viñas, J., Pérez-Serra, A., Vidal, O., Alvarado Bremer, J. R. and Pla, C. (2010). Genetic differentiation between eastern and western Mediterranean swordfish revealed by phylogeographic analysis of the mitochondrial DNA control region. *ICES Journal of Marine*, 67(6), pp. 1222 – 1229.

- Viñas J., Gordo A., Fernández-Cebrián, R., Pla, C., Vahdet, U. and Araguas, R. M. (2011). Facts and uncertainties about the genetic population structure of Atlantic bluefin tuna (*Thunnus thynnus*) in the Mediterranean. Implications for fishery management. *Reviews in Fish Biology and Fisheries*, 21, pp. 527 – 41.
- Waples, R. S. (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, 89, pp. 438 – 450.
- Waples, R. S. and Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, 15, pp. 1419 – 1439.
- Ward, R. D. (2000). Genetics of fish populations. In: Hart, P.J.B., and Reynolds, J.D., ed., *Handbook of Fish Biology and Fisheries Management*. 1<sup>st</sup> ed. United Kingdom: Blackwell Publishing, pp. 200 – 224.
- Ward, R. D., Woodward, M. and Skibinski, D. O. F. (1994). A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, 44, pp. 213 – 232.
- Ward, R. D., Elliot, N. G., Smolenski, A. J. and Grewe, P. M. (1997). Global population structure of yellowfin tuna, *Thunnus albacares*, inferred from allozyme and mitochondrial DNA variation. *Fishery Bulletin*, 95, pp. 566 – 575.
- Ward, R. D., Reeb, C. A. and Block, B. A. (2001). Population structure of Australian swordfish, *Xiphias gladius*. [online]. Final Report to the Australian Fisheries Management Authority. Available at: [http://www.soest.hawaii.edu/PFRP/pdf/afma\\_rpt.pdf](http://www.soest.hawaii.edu/PFRP/pdf/afma_rpt.pdf). [Accessed 1 February 2016].
- Witkko-Thompson, J. K., Pluzhnikov, A. and Cox, N. J. (2005). Rational Inferences about Departures from Hardy-Weinberg Equilibrium. *American Journal of Human Genetics*, 76(6), pp. 967 – 986.
- Wasser, S., Shedlock, A., Comstock, K., Ostrander, E., Mutayoba, B., Stephens, M. (2004). Assigning African elephants DNA to geographic region of origin: applications to the ivory trade. *Proceedings of the National Academy of Sciences USA*, 101, pp. 14847 – 14852.
- Wattier, R., Engel, C. R., Saumitou-Laprade, P., Valero, M. (1998). Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology*, 7, pp. 1569 – 1573.
- Weir, B. S. and Cockerham, C. C. (1984). Estimating F statistics for the analysis of population structure. *Evolution*, 38, pp. 1358 – 1370.
- West, W. M., Kerwath, S. E., Da Silva, C., Wilke, C. G. and Marsac, F. (2012). Horizontal and vertical movements of swordfish tagged with pop up-satellite transmitters in the South-West Indian Ocean, off South Africa. 10<sup>th</sup> IOTC Working Party on Billfish. *IOTC-2012-WPB10-16*. [Accessed 15 February 2016].

- West, W. M., Kerwath, S. K. (2016). The history and current status of the commercial tuna fisheries in South Africa. Unpublished.
- White, C., Selkoe, K.A., Watson, J., Siegel, D.A., Zacherl, D.C., and Toonen, R.J. (2010). Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B: Biological Sciences*, 277, pp. 1685 – 1694.
- Wirth, T. and Bernatchez, L. (2001). Genetic evidence against panmixia in the European eel. *Nature*, 409, pp. 1037 – 1040.
- Withler, R. E., Beacham, T. D., Schulze, A. D., Richards, L. J., and Miller, K. M. (2001). Co-existing populations of Pacific ocean perch, *Sebastes alutus*, in Queen Charlotte Sound, British Columbia. *Marine Biology*, 139: pp. 1 – 12.
- Wright, S. (1978). *Evolution and the Genetics of Population, Variability Within and Among Natural Populations*. The University of Chicago Press, Chicago.
- Wu, G. C. C., Chian, H. C., Chen, K. S., Hsu, C. C. and Yang, H. Y. (2009). Population structure of albacore (*Thunnus alalunga*) in the Northwestern Pacific Ocean inferred from mitochondrial DNA. *Fisheries Research*, 95, pp. 125 – 131.
- Wu, GC-C., Chiang, H-C., Chou, Y-W., Wong, Y-R., Hsu, C-C., Chen C-Y. and Yang, H-Y. (2010) Phylogeography of yellowfin tuna (*Thunnus albacares*) in the Western Pacific and the Western Indian Oceans inferred from mitochondrial DNA. *Fisheries Research*, 105, pp. 248 – 253.
- Wu, Z., Xu, Q., Zhu, J., Dai, X. and Xu, L. (2014). Genetic population structure of the bigeye tuna *Thunnus obesus* in the central Pacific Ocean based on mtDNA Cytb sequences. *Fisheries Science*, 80, pp. 415 – 426.
- Zhan, A., MacIsaac, H. J. and Cristescu, M. E. (2010). Invasion genetics of the *Ciona intestinalis* species complex: from regional endemism to global homogeneity. *Molecular Ecology*, 19, pp. 4678 – 4694.

## APPENDIX A

Table 14. A summary of population genetics studies conducted with various markers on large pelagic species and other fish species globally.

Species	Paper	Marker	Area	Species	Paper	Marker	Area
Swordfish	Alvarado Bremer <i>et al.</i> , 1995	mtDNA	Atlantic, Pacific, Mediterranean	Yellowfin tuna	Aguila <i>et al.</i> , 2015	Microsatellite	Pacific
	Alvarado Bremer <i>et al.</i> , 1996	mtDNA	Atlantic, Pacific, Mediterranean		Dammannagoda <i>et al.</i> , 2008	mtDNA, microsatellite	Indian
	Alvarado Bremer <i>et al.</i> , 1998	mtDNA	Atlantic, Pacific, Indian		Farnham, 2003	mtDNA, microsatellite	Atlantic
	Alvarado Bremer <i>et al.</i> , 2005	mtDNA	Atlantic, Pacific, Indian, Mediterranean		Kunal <i>et al.</i> , 2013	mtDNA	Indian
	Alvarado Bremer <i>et al.</i> , 2006	Nuclear	Pacific		Li <i>et al.</i> , 2015 (1)	mtDNA	Pacific
	Alvarado Bremer <i>et al.</i> , 2007	Nuclear	Atlantic, Indian		Nomura <i>et al.</i> , 2014	mtDNA, microsatellite	Pacific
	Chow and Takeyama, 2000	mtDNA, nuclear	Atlantic, Pacific, Indian, Mediterranean		Ward <i>et al.</i> , 1997	Protein, mtDNA	Atlantic, Pacific, Indian
	Chow <i>et al.</i> , 1997	mtDNA	Atlantic, Pacific, Indian, Mediterranean		Wu <i>et al.</i> , 2010	mtDNA	Pacific, Indian
	Chow <i>et al.</i> , 2002	Nuclear	Atlantic		Appleyard <i>et al.</i> , 2002	mtDNA, microsatellite	Indian
	Chow <i>et al.</i> , 2007	SNP	North and South Atlantic		Chiang <i>et al.</i> , 2006	mtDNA	Pacific
	Gardia <i>et al.</i> , 2011	mtDNA	Atlantic	Bigeye tuna	Chiang <i>et al.</i> , 2008	mtDNA	Indian
	Greig <i>et al.</i> , 1999	mtDNA, nuclear	Atlantic, North Pacific, Mediterranean		Chow <i>et al.</i> , 2000	mtDNA	Atlantic, Pacific, Indian
	Grivalja-Chon <i>et al.</i> , 1994	mtDNA	North Pacific		Durand <i>et al.</i> , 2005	mtDNA, nuclear	Atlantic, Pacific, Indian
	Kasapidis <i>et al.</i> , 2006	Microsatellite	Atlantic		Gonzalez <i>et al.</i> , 2008	Microsatellite	Atlantic, Pacific, Indian
	Kasapidis <i>et al.</i> , 2008	Microsatellite	Pacific		Wu <i>et al.</i> , 2014	mtDNA	Pacific
	Kotoulas <i>et al.</i> , 2003	Microsatellite	Atlantic, Mediterranean		Davies <i>et al.</i> , 2011	Microsatellite	Atlantic, Mediterranean
	Kotoulas <i>et al.</i> , 2006	Microsatellite	North and South Atlantic, southwest Pacific, southeast Indian and Mediterranean		Laconcha <i>et al.</i> , 2015	SNP	Atlantic, Pacific, Indian, Mediterranean
	Lu <i>et al.</i> , 2006	mtDNA	West Pacific, Indian	Albacore tuna	Montes <i>et al.</i> , 2012	Microsatellite	Atlantic, Pacific, Indian, Mediterranean
	Magoulas <i>et al.</i> , 1993	mtDNA	Northeast Atlantic, Mediterranean		Nakadate <i>et al.</i> , 2005	mtDNA, nuclear	Atlantic, Mediterranean
	Muths <i>et al.</i> , 2009	mtDNA, microsatellite	Indian		Pujolar <i>et al.</i> , 2003	Protein	Atlantic, Mediterranean
	Muths <i>et al.</i> , 2013	mtDNA, microsatellite	Indian		Takagi <i>et al.</i> , 2001	Microsatellite	Atlantic, Pacific
Marlins	Patarnello <i>et al.</i> , 2007	Review of previous studies	North Atlantic, Mediterranean	Southern bluefin tuna	Wu <i>et al.</i> , 2009	mtDNA	Pacific
	Pla <i>et al.</i> , 1998	mtDNA	Mediterranean		Grewe, 1997	mtDNA, microsatellite	Pacific
	Reeb <i>et al.</i> , 2000	mtDNA	Pacific	Atlantic Bluefin tuna	Albaina <i>et al.</i> , 2013	SNP	Atlantic, Pacific, Indian, Mediterranean
	Rosel and Block, 1996	mtDNA	Atlantic, Pacific, Mediterranean		Boustany <i>et al.</i> , 2008	mtDNA	Atlantic, Mediterranean
	Smith and Alvarado Bremer, 2010	SNP, protein	Atlantic, Mediterranean		Carlsson <i>et al.</i> , 2004	mtDNA, microsatellite	Mediterranean
	Smith <i>et al.</i> , 2015	SNP	Atlantic		Clark <i>et al.</i> , 2004	Microsatellite	Atlantic
	Vinas <i>et al.</i> , 1998	Protein, mtDNA	Mediterranean		Riccioni <i>et al.</i> , 2013	Microsatellite	Mediterranean
	Vinas <i>et al.</i> , 2006	mtDNA	North Atlantic, Mediterranean		Viñas <i>et al.</i> , 2011	mtDNA, microsatellite	Mediterranean
	Vinas <i>et al.</i> , 2010	mtDNA	Mediterranean		Dammannagoda <i>et al.</i> , 2011	mtDNA, microsatellite	Indian
	Ward <i>et al.</i> , 2001	mtDNA, microsatellite	Pacific, Indian		Schrey and Heist, 2003	Microsatellite	Atlantic, Pacific, Indian
	Graves and McDowell, 2001	mtDNA, nuclear, microsatellite	Atlantic		Beacham <i>et al.</i> , 1999	Microsatellite	Southern British Colombia
	Graves and McDowell, 2006	mtDNA, microsatellite	Atlantic		Beacham <i>et al.</i> , 2002	Microsatellite	Newfoundland, Labrador
	McDowell and Graves, 2008	mtDNA, microsatellite	Pacific		Feldheim <i>et al.</i> , 2001	Microsatellite	Western Atlantic
	Purcell <i>et al.</i> , 2011	mtDNA, microsatellite	Pacific		Schrey and Heist, 2007	Microsatellite	Missouri, Mississippi and Atchafalaya Rivers
	Sorenson <i>et al.</i> , 2013	mtDNA, microsatellite	Atlantic, Pacific		Shaw <i>et al.</i> , 1999	Microsatellite	Atlantic, Pacific



APPENDIX B

Table 15. Allele frequencies per locus for East (>20°E) and West (<20°E').

A8	Alleles	212	216	220	224	228	232	236																				
	East (>20°E)	0.003	0.003	0.04	0.857	0.079	0.016	0.003																				
	West (<20°E)	0	0	0.045	0.881	0.03	0.037	0.007																				
A7	Alleles	263	267	271	275	279	283	293																				
	East (>20°E)	0.02	0.705	0.094	0.122	0.031	0.026	0.003																				
	West (<20°E)	0.024	0.734	0.081	0.153	0.008	0	0																				
A113	Alleles	196	210	214	218	222	226	230																				
	East (>20°E)	0.003	0.116	0.077	0.082	0.54	0.173	0.009																				
	West (<20°E)	0	0.169	0.115	0.054	0.538	0.123	0																				
B6	Alleles	236	240	244	248	252	256	260																				
	East (>20°E)	0.023	0.013	0.019	0.629	0.061	0.226	0.029																				
	West (<20°E)	0.016	0	0.032	0.573	0.113	0.258	0.008																				
B108	Alleles	161	165	169	173	177	181	185	189	193	197	201	205	209	213	217	225	229										
	East (>20°E)	0.006	0.111	0.017	0.008	0.036	0.003	0.019	0.133	0.381	0.05	0.164	0.006	0.014	0.019	0.028	0	0.006										
	West (<20°E)	0	0.104	0	0.052	0	0.007	0.015	0.164	0.328	0.082	0.187	0.007	0.015	0	0.03	0.007	0										
B112	Alleles	188	192	196	200	204	208	212	216	220	224	228	232	236	240	244	248											
	East (>20°E)	0	0.006	0.006	0	0.09	0.085	0.144	0.138	0.189	0.121	0.144	0.048	0.011	0.008	0	0.008											
	West (<20°E)	0.008	0	0.025	0.008	0.107	0.082	0.172	0.164	0.189	0.074	0.115	0.049	0	0	0.008	0											
C8	Alleles	143	147	151	155	159	163	167	171	175	179	183	187	191	195	199	203	207	211	215	219	223	227	231	235	239	251	255
	East (>20°E)	0.012	0	0.006	0.054	0.03	0.048	0.057	0.131	0.077	0.11	0.068	0.068	0.08	0.054	0.039	0.045	0.036	0.009	0.003	0.018	0.018	0.012	0.009	0.012	0.003	0.003	0
	West (<20°E)	0	0.008	0.023	0.039	0.031	0.062	0.039	0.141	0.047	0.102	0.07	0.102	0.062	0.047	0.039	0.039	0.016	0.031	0.031	0.031	0	0.008	0	0.016	0.008	0	0.008
C10	Alleles	183	187	191	195	199	203	207	211	215	219	223	227	231	235	239	243	247	251	255	267	271	279					

	East (>20°E)	0.003	0.009	0.021	0.034	0.034	0.043	0.046	0.181	0.11	0.132	0.107	0.058	0.074	0.071	0.04	0.021	0.006	0	0.003	0	0.003	0.003
	West (<20°E)	0.008	0.008	0.033	0.041	0.025	0.057	0.115	0.156	0.115	0.09	0.115	0.057	0.041	0.041	0.041	0.016	0.016	0.008	0.008	0	0.008	0
D2B	Alleles	147	155	159	163	167	171	175	179	183	187	191											
	East (>20°E)	0.003	0.017	0.006	0.097	0.287	0.34	0.171	0.052	0.014	0.011	0.003											
	West (<20°E)	0	0	0	0.101	0.283	0.348	0.167	0.087	0.007	0.007	0											
Xg166	Alleles	111	114	120	123	126	129	132	135	138	141	144											
	East (>20°E)	0.006	0.006	0.022	0.003	0.157	0.091	0.569	0.088	0.033	0.022	0.003											
	West (<20°E)	0	0	0.031	0	0.115	0.108	0.523	0.192	0.015	0.015	0											
Xg144	Alleles	110	119	125	128	131	134	137	140	152	155	158	161	164	167	170							
	East (>20°E)	0.003	0.009	0.032	0.019	0.098	0.013	0.006	0.003	0.003	0.019	0.351	0.351	0.082	0.006	0.003							
	West (<20°E)	0.018	0.088	0.009	0.018	0.061	0	0	0	0	0.009	0.342	0.281	0.158	0.018	0							

## CHAPTER 3

### **Abundance of swordfish (*Xiphias gladius*) based on standardised catch rates in the South African pelagic longline fleet (1998 – 2014)**

#### **Abstract**

Catch-per-unit-effort (CPUE) of swordfish (*Xiphias gladius*) derived from catch and effort data from the South African pelagic longline fleet operating in the South Atlantic and Indian oceans was standardised with generalised linear models (GLMs). An admixture zone may exist between 15°E and 27°E where the swordfish stocks of the South Atlantic Ocean and Indian Ocean meet. The current management boundary at 20°E means that both stocks have swordfish from the neighbouring stock in its jurisdiction. The effect of this admixed area on abundance trends is unknown. The CPUE standardisation models were run with the admixed data included, and excluded, in the South Atlantic Ocean (<20°E) and the Indian Ocean (≥20°E). The tweedie model with a Poisson distribution and a log-link function was chosen to model the catch in number of fish/1000 hooks<sup>-1</sup>. Cluster analysis categorised fishing effort into different fishing tactics based on similarity in the catch composition. Other factors included in the model were season, sea surface temperature (SST), vessel length, latitude, longitude and number of hooks as an offset term. Total deviance explained by the model was 49.21% and 61.95% for the South Atlantic and Indian Ocean, respectively, of which year & season and year & vessel length explained a majority of the variation. The standardised CPUE trends indicated a peak in Swordfish abundance at the start of the fishery followed by a steady decline since. Fishing effort focused within and around the 200 nm exclusive economic zone (EEZ) and may have resulted in localised depletion of this species. There were no qualitative differences in the abundance trends of the models that included the admixed zone with those that did not. Therefore, at the current management boundary the inclusion of abundance indices from South Africa should not be discarded on account of stock origin concerns in this area.

#### **Introduction**

Stock assessment involves the use of various statistical and mathematical calculations to make quantitative predictions about the reactions of fish populations to alternative management choices (Hillborn and Walters, 1992). Stock assessment techniques generally involve a variety of research and monitoring data inputs to estimate parameters (*e.g.* abundance) that are of interest to decision makers (Maunder and Punt, 2004). A routine step in conducting a stock assessment is to estimate the change in relative abundance of a fish stock over time. The change in relative abundance does

not reflect the actual biomass of the stock but rather the rate at which it has increased and decreased.

Catch rate or CPUE is under certain circumstances assumed to be proportional to abundance. Catch is proportional to the product of fishing effort and density:

$$C = qEB \quad (1)$$

where  $E$  is the fishing effort expended,  $B$  the mean population biomass, and  $q$  the fraction of the abundance that is captured by one unit of effort (often referred to as the catchability coefficient) (Maunder and Punt, 2004). The catch rate is derived from this equation:

$$\frac{C}{E} = qD = q\frac{N}{A} \quad (2)$$

where  $N$  is the number of fish of the fishing grounds and  $A$  the area of the fishing grounds.

In a stock assessment, in simple terms, the CPUE-based relative abundance is used to calibrate the relative abundance predicted by the stock assessment (through data on mortality and recruitment to determine absolute abundance) and the stock assessment model parameters such as  $r$  (population increase) and  $K$  (carrying capacity) are determined through maximum likelihood estimation to fit to the CPUE index (Hinton and Maunder, 2003).

The key to producing a reliable index of abundance is consistency, regardless of how many more fish one index contains than another index (Cooper, 2006).  $q$  Should remain constant for comparability over time and to analyse data from large fisheries with many vessels (Marchal *et al.*, 2003). The influence of factors such as time, space and fishing fleet, vessel and gear characteristics should be removed. When  $q$  is not constant then it is necessary to remove the impacts that those changes have on the catch rate that are not due to changes in abundance (Marchal *et al.*, 2003). This is achieved by applying a fixed standard set of factors and predict the index of abundance for each year. This procedure is referred to as standardising the CPUE.

Data on catch and effort can either be obtained from fishery-independent research surveys with standardised gear and coverage of the entire fishing ground to determine range contractions or expansions (Cooper, 2006). Fishery-independent data are consistent and ideal for estimations of

relative abundance. Unfortunately, for species that span large areas (*e.g.* tuna, billfish and shark), it is impractical to conduct fishery-independent surveys and the alternative is to rely on simple and inexpensive fishery-dependent catch and effort data. However, fishery-dependent CPUE estimates will violate the assumptions of consistency. Fishermen will fish in areas of known fish abundance influenced by past catches and/or through reading environmental conditions presently. This can falsely elevate or maintain CPUE indices, referred to as hyper-stability, even though the abundance is on the decline (Hillborn and Walters, 1992). Conversely hyper-depletion can falsely lower CPUE indices when effort is concentrated in areas of low abundance yet the abundance of the stock unit is stable or on the increase (Hillborn and Walters, 1992). In addition, fishermen will also change and modify gear as technology improves. The change in target due to access to markets, quota availability or quota limitations will also change the catchability.

Standardised CPUEs have an application in stock assessments of a wide variety of harvested species worldwide. Over the years there have been considerable improvements of the techniques to standardise CPUE (*e.g.* Lancia *et al.*, 1996; Campbell, 2004; Maunder and Starr, 2003; Maunder and Punt, 2004; Venables and Dichmont, 2004; Lynch *et al.*, 2012; Campbell, 2015) with methods including generalised linear models (GLMs), generalised additive models (GAMs), generalised linear mixed models (GLMMs) and regression trees. There has also been considerable discussion on which of the many error distributions to choose from (*e.g.* normal, gamma, Poisson, negative binomial and tweedie distributions) and which of these may be the most appropriate to use with these methods (Campbell, 2015). In conjunction with research on techniques is research on the relationship between standardised CPUE and relative abundance and how to address factors that influence this relationship. Walters (2003) discussed two important issues related to spatial parameters; 1) the summing of catch and effort data over large spatial cells to produce one ratio of relative abundance (the folly) and 2) unfished spatial cells are ignored (the fantasy). Walters (2003) emphasised the importance of averaging catch per effort data spatially and temporally in areas and during years that were unfished to avoid scenarios of hyper-depletion and hyper-stability.

Preparing the data for the model is the first important step before the model type and error distribution is decided on. Factors to consider include addressing changes in targeting, anomalous values, the number of variables to include avoiding over fitting or under fitting the model etc. Data preparation will influence model outcomes considerably. Despite the criticisms and challenges of the use of standardised CPUE time series as abundance index, there are few alternatives in the absence of fishery-independent data.

The necessity to standardise catch rates has been known for many years and over the years many methods have been proposed and implemented. The most common method prior to the use of generalised linear models was that of Beverton and Holt (1957) where vessel power for a fleet was measured in relation to a standard vessel (Maunder and Punt, 2004). Beverton and Parrish (1956), Gulland (1956) and Robson (1966) applied similar simple methods to adjust effort to account for changes in catchability. However, multiple factors, not only vessel power, influence catch rates. Gavaris (1980) and Kimura (1981) were first to fit statistical models to the data with generalised linear models (Maunder and Punt, 2004). There has been steady stream of publications on statistical techniques to standardise CPUE, such as Maunder and Punt (2004), Tascheri *et al.* (2010), Cao *et al.* (2011), Lynch *et al.* (2012) and Brodziak and Walsh (2013) (in Campbell, 2015). These techniques cover a range of methods including GLMs, GAMs, GLMMs and regression trees (Campbell, 2015). GAMs are an extension of GLMs that replace the linear predictor with a smoother function (such as a spline or loess smoother) (Maunder and Punt, 2004). GLMMs are a further and more fundamental extension of the GLM by allowing some of the parameters in the linear predictor to be treated as random variables (Maunder and Punt, 2004).

### GLMs

GLMs were first formulated by John Nelder and Robert Wedderburn (1972) and express the relationship between a response variable,  $Y$ , and a number of covariates or explanatory variables (categorical or continuous),  $X$ . The GLM is made up of a linear predictor and two functions (link and variance functions). The GLM allows the response variable to have an error distribution model that is not a normal distribution. “Generalised” of GLM refers to the dependence on potentially more than one explanatory variable, vs. the simple linear model.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots \beta_k x_k + \epsilon \quad (3)$$

where  $Y$  is the response variable,  $\beta_0$  to  $\beta_k$  are constants and occur in linear fashion (being estimated),  $x_1$  to  $x_k$  are independent variables without error and  $\epsilon$  is a random error.

The covariates have a combined linear effect on  $Y$ . Each component of  $Y$  is independent and is from one of the exponential family of distributions. The relationship between the mean of  $Y$  and the linear covariates is specified via a link function:

$$g(\mu_i) = \alpha + X_i\beta \quad (4)$$

where  $\mu_i$  is the mean response variable on the  $i^{\text{th}}$  row.  $X_i\beta$  is the linear predictor:  $X_i$  is the vector of covariate variables for the  $i^{\text{th}}$  row and  $\beta$  the vector of coefficients associated with the covariates.

Hence:

$$\mu_i = g^{-1}(X_i\beta) \quad (5)$$

The link function chosen is dependent upon the probability distribution of the response variable. The link function must be differentiable and monotonic (either strictly increasing or strictly decreasing), such as Identity, Log, Logit and Reciprocal (Anderson *et al.*, 2004). The variance function describes how the variance depends on the mean:

$$V(Y) = \phi V(\mu) = V(g^{-1}(X_i\beta)) \quad (6)$$

$\phi$  is a constant dispersion parameter. The unknown parameters,  $\beta$ , are typically estimated with maximum likelihood, maximum quasi-likelihood, or Bayesian techniques. The maximum likelihood estimates can be found with an iteratively reweighted least squares algorithm (Jennrich and Sampson, 1976).

Interaction terms may be included in the model. An interaction occurs when an independent variable has a different effect on the outcome depending on the values of another independent variable.

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3(x_1 * x_2) \quad (7)$$

where  $\beta_3(x_1 * x_2)$  is the interaction term. The main objective of standardising the CPUE is to estimate the effect of the variable ‘year’. Interactions with the year effect would invalidate the year effect as an index of abundance. For this reason, most analyses do not consider interaction terms for the year effect (Hinton and Maunder, 2003). When the effect of an explanatory variable is known, it is appropriate to include information about this variable in the model as a known effect, by introducing an offset term  $\xi$ , such as hook number, into the definition of the linear prediction (Anderson *et al.*, 2004), giving:

$$\mu_i = g^{-1}(X_i\beta + \xi) \quad (8)$$

#### *Recent abundance index models applied to pelagic longline fishing species*

Pelagic longline gear is a passive method of fishing since the line is left to soak in the water and attract fish to the hooks. This method of fishing can produce zero catch of the intended targeted species if this species does not hook on, for various reasons. The analysis of a covariance model with a normal error structure cannot be applied to the “zero-catch” data because the natural logarithm of zero is equal to negative infinity. Although previous studies have explored alternate methods to handle observations with zero catch, the lognormal and the delta lognormal GLM models remain popular to standardise the catches of pelagic longline species. When Regional Fisheries Management Organisations (RFMOs) conduct stock assessments, Contracting Party Countries (CPCs) submit abundance indices for their fleets. The CPC with historical time series of data and/or constitute high proportions of the pelagic longline effort are encouraged to submit abundance indices. In recent years the lognormal (Bigelow *et al.*, 2004; Yeh and Chang, 2013; Chang and Yeh, 2014; Lee *et al.*, 2014; Matsumoto *et al.*, 2014; Ochi *et al.*, 2014; Okamoto, 2014; Ijima *et al.*, 2015; Matsumoto *et al.*, 2015; Matsumoto and Satoh, 2015; Ochi, 2015; Yeh and Chang, 2015), delta lognormal (Wang and Nishida, 2013a; Wang and Nishida, 2013b; Pons and Domingo, 2014; Laretta and Walter, 2015; Walter, 2015; Wang, 2015) and negative binomial (Kiyofuji, 2014; Matsumoto, 2014; Okamoto and Ijima, 2015) models have been utilised for bigeye tuna (*Thunnus obesus*), yellowfin tuna (*Thunnus albacares*), albacore (*Thunnus alalunga*), skipjack tuna (*Katsuwonus pelamis*), striped marlin (*Kajikia audax*), blue marlin (*Makaira nigricans*) and sailfish (*Istiophorus albicans*) in the Atlantic and Indian oceans.



### *Trends in swordfish abundance indices*

In the Indian Ocean, the trends in the abundance indices differ among fleets and the increasing and decreasing trends occur at different time periods (Semba *et al.*, 2008). The area coverage, model choice and parameter choice differ among fleets, making it challenging to find a complete and true representation of how the abundance of the Indian Ocean stock has changed. The Japanese fleet has experienced a decrease in abundance from 1971 to 2005, with increases 2006 onwards (Nishida and Wang, 2014). The Portuguese pelagic longline fleet has reported an increasing trend for the whole of the Indian Ocean. In the South West Indian Ocean (SWIO) region, on the other hand, this fleet has indicated a general decrease for the more recent years (Santos *et al.*, 2013; Santos *et al.*, 2014b). In contrast to the two above mentioned fleets, Taiwan's fleet shows no clear trend in every area specific standardised CPUE model (Wang and Nishida, 2014)

In the South Atlantic, the abundance indices of Taiwan (Chang *et al.*, 2007; Sun *et al.*, 2014) and Uruguay (Pons *et al.*, 2014) indicate recent decreasing abundance trends, whilst the Japanese (Kai and Yokawa, 2014) have reported increased trends since 2000 and Brazil (Hazin *et al.*, 2014) report an increasing recent until recent years. In contrast to the aforementioned fleets, the results from the Spanish (Ramos-Cartelle *et al.*, 2014) indicate a very stable standardised CPUE.

### *South Africa's fisheries data: history and nuances to consider for the CPUE*

From the start of the experimental pelagic longline fishery, a minimum size limit of 125 cm lower jaw fork length (LJFL) and 25 kg (whole weight) was enforced until 2005, when that was reduced to 119 cm LJFL and 18 kg (dressed weight) to minimise dumping at sea. All undersize swordfish were confiscated by the Fishery Control Officers/Monitors who were required to monitor all discharges of longline vessels fishing on a South African permit.

During the experimental phase of the fishery (1997 – 2004) South Africa established a self-imposed quota limit of 1000 t until 2002. South Africa was excluded from the sharing arrangement for south Atlantic swordfish and had not been granted a swordfish allocation for the International Commission for the Conservation of Atlantic Tuna (ICCAT) convention area. A further restriction on all vessels from 1997 – 2004 was that swordfish may not exceed 15% of the total catch per landing. The intention of these restrictions was to prevent the development of a swordfish fishery in the ICCAT convention area. This by-catch limit in the South African EEZ during 1998 and 1999 had forced many

of the South African fishers to land their catches in ports of neighbouring countries. Furthermore, and very importantly, it was later discovered through import statistics from the United States that the 15% by-catch limit on swordfish resulted in vessels declaring swordfish catch as tunas (bigeye and yellowfin). Without properly established monitoring and compliance at the start of the fishery this misreporting and underreporting went unnoticed.

From 2003 onwards ICCAT issued South Africa catch quotas for the Atlantic Ocean-caught swordfish. The South African quota limits have ranged between 890t and 1200t and the current quota is 1001t for 2015. South Africa has never been limited by this quota and the annual catches in most recent years are around 200t.

Since 2005, the foreign-flagged joint venture vessels have had a swordfish bycatch limit of 15% of the total weight of catch in South Africa's EEZ in the Indian Ocean. On average, swordfish is 3% of the total weight landed by these vessels, making this a non-limiting factor. In the Atlantic Ocean, these vessels have had unrestricted access to swordfish until 500t have been caught, but that threshold has not been met. South African vessels have had unrestricted access to swordfish in the Indian oceans since 2005.

### *Chapter aim*

The aim of this chapter is to investigate the swordfish abundance around South Africa from two stocks, South Atlantic and Indian Ocean, by standardising the catch-per-unit-effort of the pelagic longline fleet from 1998 to 2014 to create an index of relative abundance.

Pelagic longline data from 1997 to 2014 will be extracted with queries from a MS Access database that is owned and managed by the Department of Agriculture, Forestry and Fisheries (DAFF). The data will be filtered in MS Excel and variables other than swordfish abundance that could affect catchability will be chosen, along with a GLM model with the appropriate link function and error distribution. The model will predict the annual change in relative swordfish abundance when the effects of the variables have been removed. The change in relative abundance of swordfish will be discussed in relation to fishery characteristics and the species biology.

## Materials and Methods

### *Fishing area*

Longline sets were distributed across the Atlantic and Indian oceans around South Africa, ranging from 0°E to 45°E longitude and 23°S to 40°S latitude from 1998 to 2014 (Figure 1). The fishing sets are recorded in a 1° x 1° resolution. The South Atlantic and Indian Ocean swordfish stocks are currently separated by a 20°E management boundary line that does not account for the likely admixture in this area. The oceanographic characteristics around South Africa may influence the movement and range of the stocks directly or indirectly (i.e. food availability). The Agulhas current follows the continental shelf break of south-eastern Africa closely (Grundlingh, 1983) until it reaches the southern tip of Africa where it retroflects back into the South Indian Ocean. The Agulhas Return Current lies on average at a latitude of 39°30' S south of Africa (Lutjeharms and Ansorge, 2001). The Benguela current is part of the South Atlantic Ocean gyre that flows northward from around Cape Point 35°S to around 16°S and forms coastal upwelling called the Benguela Upwelling System (Andrews and Hutchings, 1980).

The population genetics results of the previous chapter indicated mixed stocks of swordfish between, though not limited to, 14°E and 27°E. The stock origin of swordfish in this region is uncertain and the mixing ratio of Indian Ocean and South Atlantic stocks has not been determined in this region. It was decided to run comparative models with this admixture zone included, and excluded, to check for the effect of including data containing mixed stocks of swordfish. Therefore, for the South Atlantic Ocean/West area model one contained all data <20°E of the stock boundary and model two contained data ≤15°E. And for the Indian Ocean/East area model one contained all data ≥20°E of the stock boundary and model two contained data ≥27°E.

### *Fisheries Catch and Effort Data*

The vessels have been required to fill in logbooks onboard containing fields such as catch in number and weight, Global Positioning System (GPS) catch location of setting and hauling, time of setting and hauling, bait used, as well as branch line length. Since this is a relatively new fishery, refining the type of data collected is an ongoing process and additional fields of data have been included to facilitate analyses. Catch data were obtained from 29 281 longline sets of all pelagic longline vessels operating in the Large Pelagics Longline sector. Sets that were made in the first year of the experimental fishery in 1997, sets that contained missing catch location and number of hooks and

sets of vessels that traditionally target pelagic sharks (i.e. these vessels had an average of 1.6% of the total weight caught was swordfish) were removed from the dataset (Table 1). Vessels that had been fishing for fewer than 4 years and/or had made fewer than 200 sets were removed from the dataset, leaving 31 vessels in the dataset. By excluding these vessels, the variation from intermittent fishing over short periods has been minimised (Table 2).

Overall, 31.84% of the data was removed (Table 1) and the analysis continued with 6730 longline sets in the South Atlantic Ocean model one ( $<20^{\circ}\text{E}$ . Referred to as West ( $<20^{\circ}\text{E}$ )), 3661 longline sets in the South Atlantic Ocean model two ( $\leq 15^{\circ}\text{E}$ . Referred to as West ( $\leq 15^{\circ}\text{E}$ )), 13228 longline sets in the Indian Ocean model one ( $\geq 20^{\circ}\text{E}$ . Referred to as East ( $\geq 20^{\circ}\text{E}$ )), 7252 longline sets in the Indian Ocean model two ( $\geq 27^{\circ}\text{E}$ . Referred to as East ( $\geq 27^{\circ}\text{E}$ )). The distribution of average nominal CPUE and average number of hooks set, after filtering criteria had been applied, indicating that nominal swordfish CPUE is highest West of Cape Town and East of Richards Bay (Figure 1) although the effort is concentrated along the South East coast and North West of South Africa (Figure 2). The difference in effort concentrations and nominal CPUE may be attributed to effort dedicated towards tuna targeting.

Table 1. The number and percentage of records removed and the reason for removal from the dataset.

<b>Filtering criteria</b>	<b>Reason for removal</b>	<b>Number of records removed</b>	<b>% data removed</b>
Catch made in 1997	Minimal catches made at the start of the experimental fishery	10	0.03
Catch made by pelagic shark targeting vessels incorporated in the Large Pelagic Longline fishery in April 2011.	Swordfish is a bycatch species, making up on average 1.6% of the total catch.	1278	4.36
Missing catch location	The catch cannot be assigned to a stock	293	1.00
Missing hook number	A catch rate cannot be calculated	25	0.09
Vessels operating for <4 years and/or have made <200 sets	Minimise the variation from intermittent fishing over short periods and low experience from few sets made	7717	26.35
<b>Total</b>		<b>9323</b>	<b>31.84%</b>

Table 2. Summary of number of sets made per year, per vessel and number of years active per vessel.

Vessel	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total number of sets per vessel	Total number of years active per vessel
Vessel 1				27		71	106		20	125	128	132						609	7
Vessel 2			65	65	105	83		10	11									339	6
Vessel 3			59	34	49	36	110	52										340	6
Vessel 4	42	18	24	62	85	69	100	77	71	74	77	56						755	12
Vessel 5				41	137	118	118	113	165	173	111	125	185	173	141	156	125	1881	14
Vessel 6	12	14	36	128	136	100	18											444	7
Vessel 7				35	121	123	116											395	4
Vessel 8	4	81	66	96														247	4
Vessel 9						120	70	91	96	49								426	5
Vessel 10					65	103	35	58	22									283	5
Vessel 11	57	61	78	1		78		84		79								438	7
Vessel 12												58	162	138	148	147	146	799	6
Vessel 13										110	169	186	195	122	108	129		1019	7
Vessel 14										91	147	117	173	113	90	135	123	989	8
Vessel 15												94	132	119	43	90	101	579	6
Vessel 16			11	87	116	53	9	73	43	25			12	122	98	63	41	753	13
Vessel 17			50	131	159	101												441	4
Vessel 18										48	101	102	63	134	152	173	143	916	8
Vessel 19							59	86	154	136	104	123	75	136	103	110	56	1142	11
Vessel 20											86	57	123	118	88	56	94	622	7
Vessel 21												82	111	107	65	120	116	601	6
Vessel 22											39		64	84	87	117	116	507	6
Vessel 23	21	30	33	79	59	15					10	45	122					414	9
Vessel 24										113	154	144	150	112	89	118		880	7
Vessel 25										150	100	169	95	89	39			642	6
Vessel 26	5	8	156	83	83	106												441	6
Vessel 27										36	93	121	162	87	130	132	76	837	8
Vessel 28												83	118	129	86	93	88	597	6
Vessel 29													168	144	135	150		597	4
Vessel 30										81	40			89	48	142		400	5
Vessel 31												109	148	148	75	145		625	5
<b>Total number of sets per year</b>	<b>141</b>	<b>212</b>	<b>578</b>	<b>869</b>	<b>1115</b>	<b>1176</b>	<b>741</b>	<b>644</b>	<b>582</b>	<b>1290</b>	<b>1359</b>	<b>1803</b>	<b>2258</b>	<b>2164</b>	<b>1725</b>	<b>2076</b>	<b>1225</b>		

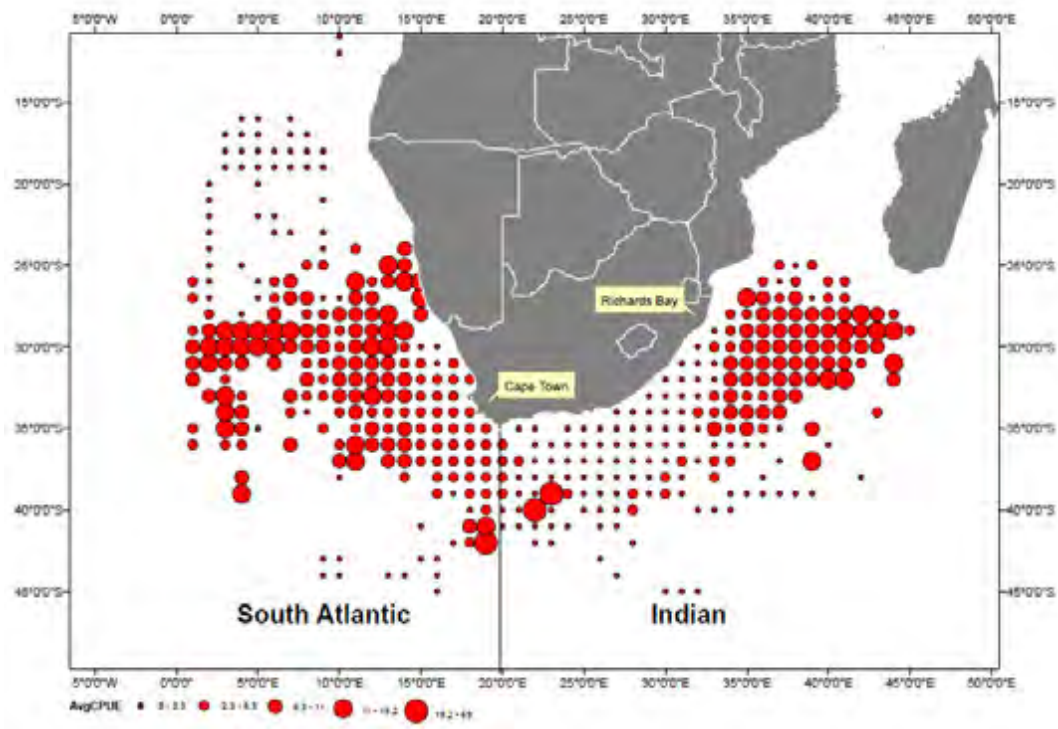


Figure 1. Average swordfish nominal CPUE from 1998 – 2014 for the entire South African coastline after filtering criteria have been applied. The line at 20°E demarcates the management boundary between ICCAT and IOTC.

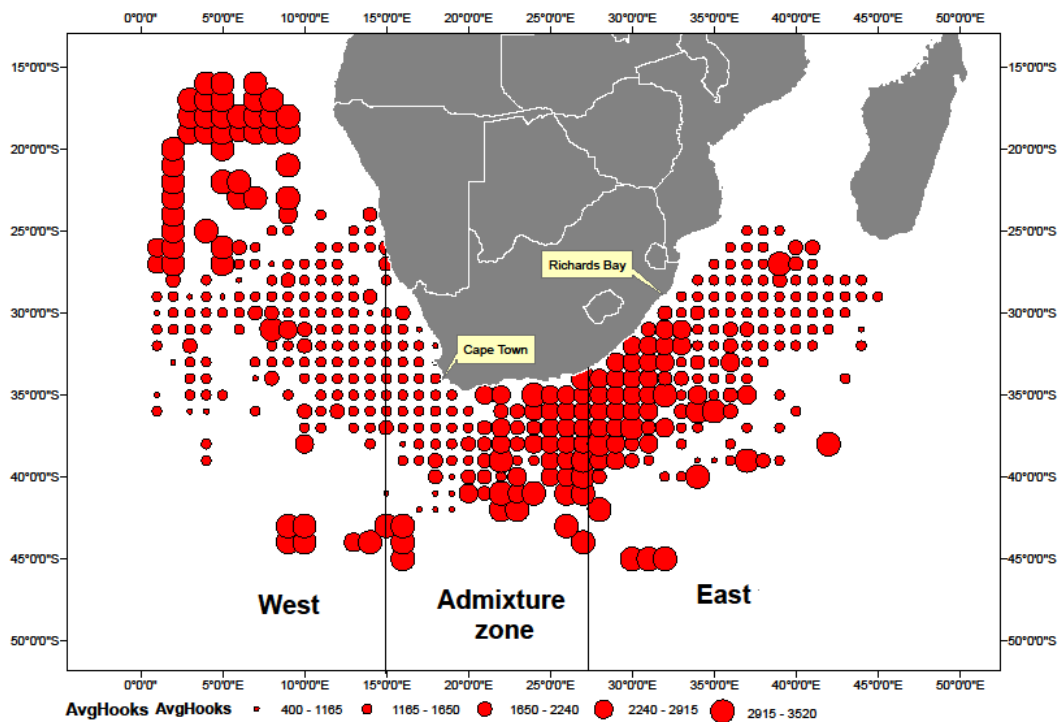


Figure 2. The geographic distribution of longline sets from 1998 – 2014 for the entire South African coastline after filtering criteria have been applied. The admixture zone between Atlantic and Indian Ocean stocks is indicated.

### *Cluster analysis*

Clustering methods (*e.g.* cluster analysis) have been applied in the analysis of fishing data, aiming at categorising fishing efforts based on the similarity in catch composition, as a way to detect differences in targeting. The fishing tactic can be included in the standardisation model as a categorical variable to adjust for differences in catchability associated with each cluster (*e.g.* Hazin *et al.*, 2006; Pelletier and Ferraris, 2000; Carvalho *et al.*, 2010; Deporte *et al.*, 2012; Winker *et al.*, 2013; Winker *et al.*, 2014). The k-means method (MacQueen, 1967), where each cluster is represented by the centre of the cluster, was chosen for the clustering analysis. There were 19 species that had consistent presence (present in catches for 12 years or more over the 1998 to 2014 time period) in the fishery that were included in the dataset (Table 3). The optimal number of clusters per model was predicted with the *prediction.strength* function (Tibshirani and Walther, 2005) in the R package 'fpc'. The function chose the largest number of clusters that leads to prediction strength above 0.8. The software program Cluster 3.0 (Eisen *et al.*, 1998; de Hoon *et al.*, 2004) was used to assign the pelagic longline sets into fishing tactics with the *k*-means clustering algorithm.

### *Data analysis*

All data preparation was conducted in Microsoft (MS) Excel 2010 and all statistical analyses were performed in RStudio Desktop v0.98.501 within the statistical programme language R project for Statistical Computing v3.1 (R Core Team, 2015). The maps were created in ArcMap in ArcGIS 10.1. The world shapefile was obtained from the Thematic mapping website (Sandvik, 2013).

### *Model choice and parameters*

Shono (2008) compared the statistical performance of four models (Negative Binomial model, the ad-hoc method, Delta-lognormal and Tweedie distribution) on data with a large proportion of zero-catch ( $>2/3^{\text{rd}}$ ) and with a small proportion of zero-catch ( $<1/3^{\text{rd}}$ ), to find the most suitable model to overcome the zero-catch problem. Carvalho *et al.*, (2010) conducted a similar study comparing the model performance of the negative binomial, delta-lognormal and Tweedie distribution on blue sharks caught in the south western Atlantic Ocean Model performance. The results of both studies indicated a superior statistical performance by the Tweedie distribution model. The Tweedie distributions, named by Bent Jørgensen (1987) after Maurice Tweedie, are a family of probability distributions which include the purely continuous normal and gamma distributions, the purely



Table 3. The percentage catch contribution towards the total weight caught per year of species included in the clustering analysis dataset.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Bigeye tuna ( <i>Thunnus obesus</i> )	7.4	7.7	13.4	8.9	19.1	13.5	20.5	20.6	11.4	13.4	15.0	15.7	16.6	11.0	14.7	13.9	8.6
Yellowfin tuna ( <i>Thunnus albacares</i> )	15.5	33.6	32.5	9.6	7.0	18.9	33.3	42.1	25.0	30.3	17.7	36.7	44.5	23.2	17.8	24.4	12.1
Albacore ( <i>Thunnus alalunga</i> )	9.6	15.9	23.3	25.9	21.3	15.4	12.2	12.2	21.1	15.7	23.3	21.1	10.4	12.6	14.3	14.4	6.8
Southern bluefin tuna ( <i>Thunnus maccoyii</i> )	0.1	0.1	0.2	0.1	0.5	0.4	0.4	0.3	0.4	0.8	0.8	0.4	0.5	0.4	1.0	0.4	0.4
Unidentified tuna	3.8	0.5	0.7	0.1	0.4	0.5	2.0	0.6	0.0	2.0	1.2	0.1	0.2	0.3	0.4	0.0	0.0
Swordfish ( <i>Xiphias gladius</i> )	45.8	19.1	11.7	23.8	28.7	20.9	10.4	6.0	18.3	8.1	7.3	6.4	8.5	4.9	5.5	4.8	2.4
Black marlin ( <i>Istiompax indica</i> )	0.0	0.0	0.0	0.0	0.1	0.3	0.0	0.0	0.1	0.0	0.2	0.1	0.4	0.2	0.1	0.1	0.0
Blue marlin ( <i>Makaira nigricans</i> )	0.0	0.2	0.1	0.0	0.2	0.1	0.1	0.0	0.1	0.1	0.2	0.0	0.1	0.1	0.1	0.1	0.0
Unidentified billfish species	0.0	0.0	0.0	0.1	0.6	0.4	0.2	0.5	0.0	0.1	1.6	0.1	0.3	0.2	0.1	0.0	0.0
Sailfish ( <i>Istiophorus albicans</i> )	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.0
Mako shark ( <i>Isurus</i> spp.)	2.6	2.6	4.0	4.0	2.0	3.2	3.1	2.2	3.2	2.8	2.5	3.5	3.0	12.1	9.3	13.2	16.8
Blue shark ( <i>Prionace glauca</i> )	3.5	7.7	5.3	16.0	9.4	12.5	8.5	7.0	7.8	7.3	8.2	4.0	4.2	23.1	22.6	20.4	48.7
Unidentified sharks	0.7	0.4	1.2	1.6	0.5	0.5	0.5	0.1	1.2	0.2	0.6	0.2	0.1	0.1	0.1	0.0	0.0
Other fish	0.0	0.1	0.4	0.8	0.3	0.6	3.2	0.0	0.0	0.3	0.0	0.1	0.3	1.8	1.8	0.1	0.0
Dolphin fish ( <i>Coryphaena hippurus</i> )	0.0	0.0	0.0	0.4	0.7	1.6	0.3	0.3	1.4	0.8	1.5	0.7	1.9	1.1	1.8	1.0	0.8
Oilfish ( <i>Ruvettus pretiosus</i> )	0.0	0.5	2.7	4.5	6.6	8.8	2.8	3.8	2.5	14.3	10.6	6.1	4.7	4.7	5.5	2.5	1.0
Escolar ( <i>Lepidocybium flavobrunneum</i> )	0.0	0.0	0.1	0.4	0.1	0.1	0.0	2.5	1.1	1.9	6.6	2.5	0.9	2.8	1.8	2.4	0.8
Wahoo ( <i>Acanthocybium solandri</i> )	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0
Moonfish ( <i>Lampris</i> spp.)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5	0.1	1.7	0.4	1.8	0.8	0.2

discrete scaled Poisson distribution, and the class of mixed compound Poisson–gamma distributions which have positive mass at zero, but are otherwise continuous (Tweedie, 1984).

The Tweedie distribution model can handle zero-catch uniformly (Tweedie, 1984). The model is defined by three parameters,  $\mu$ : location parameter;  $\sigma^2$ : diffusion parameter;  $p$ : power parameter; and variance =  $\sigma^2 \mu^p$ . When the power parameter is in the range  $1 < p < 2$ , it takes the form of the Poisson distribution and it is able to model zero-catch data appropriately (Shono, 2008).

The response variable for the model is CPUE measured in number of fish per 1000 hooks (nr/1000 hooks<sup>-1</sup>). The number of fish is a more accurate expression of catch since there are a finite number of hooks set into the water, in contrast to a net-based method that is limited by the weight of fish that can fit into the net.

The details for the explanatory variables chosen for the models are as follows:

1. *Season*

The pelagic longline fishing season is protracted over two to three months for some target species over the year. This is evident by the trips of the freezer vessels that last for 3 – 6 months to maximise on the availability of the large pelagic species in the area. Therefore, the temporal factor was classified into four seasons, summer (Nov - Jan), autumn (Feb - Apr), winter (May - Jul), spring (Aug – Oct).

2. *Sea surface temperature (SST):*

Large pelagic species generally have a wide temperature tolerance, evident by their ability to conduct regular dives to depths in excess of 600 m. The SST was classified into two main categories to distinguish a temperate condition from a more tropical environment which may have influenced the presence of the large pelagic species or the prey on which they feed. The SST categories are 2-12°C, 13-24°C and >24°C.

3. *Vessel length overall (LOA):*

The vessel length indicates the flag, the fishing range and holding capacity. The larger vessels are foreign-flagged vessels that were in bilateral or joint-venture agreements with South Africa. The fishing style of these vessels was geared towards tuna targeting. The smaller vessels are limited in the number of fish they can retain and the fishing areas they can access. The LOA was categorised into 18-25 m, 26-35 m, and 36-44 m.

4. *Fishing tactic*:

This is based on the outcome of the cluster analyses. The foreign-flagged vessels target tunas and the local vessels target a combination of swordfish, tunas and sharks. The fishing tactic may have a large influence on the catch rates of swordfish.

5. *Latitude and Longitude*:

The fishing area in this study is small in relation to the range of the large pelagic stocks that span the entire Indian Ocean and South Atlantic Ocean. It was not deemed necessary to divide the area in this study into sub-areas. Hence the latitude and longitude were included in 1°x1° blocks to detect patterns on a smaller scale.

6. *LOA\*Latitude and LOA\*Longitude* interaction term:

The larger vessels may be accessing fishing grounds that are further offshore.

7. *Season\*SST* interaction term:

There may be seasonal changes in SST that can affect the availability of swordfish in the area.

8. *Number of hooks* included as an offset term :

The number of hooks is a known effect, the more hooks set the greater the chance of catching more fish, and was included as an offset term.

Year, area, season, SST, LOA, fishing tactic are defined as categorical variables, with latitude, longitude and number of hooks defined as a continuous variable. The Tweedie distribution model was run in R (R Core Team, 2015) with the “tweedie” package (Dunn, 2014) and “statmod” package.

*Model procedure*

The Tweedie model is able to express Poisson, Gamma and inverse Gaussian distributions if the power parameter,  $p$ , is 1, 2 and 3, respectively (Shono, 2008). The Poisson distribution is appropriate for a high prevalence of zero catch data. A  $p$  value  $1 < p < 2$  was estimated by maximising the profile log-likelihood.

Because the main objective of CPUE analyses is to extract the annual trend in relative abundance, the main effect of year is included into the null model (Shono, 2008) (Eq.9). The full model is included in Eq.10.

$$CPUE = Intercept + Year_i + Error_i \quad (9)$$

$$CPUE = Intercept + Year_i + Season_j + SST_k + LOA_l + Tactic_m + LAT_n + LONG_o + (LOA * LAT)_{ln} + (LOA * LONG)_{lo} + (SEASON * SST)_{jk} + offset(Hook) + Error_{ijklmn} \quad (10)$$

where *SST* is sea surface temperature, *LOA* is vessel length overall (m), *LAT* is latitude, *LONG* is longitude. The tweedie model with a Poisson distribution and log-link function was chosen to standardise the CPUE.

The Akaike Information Criterion (AIC) (Akaike, 1973) was modified and uses the quasi-likelihood constructed from the estimating equations (Wedderburn, 1974; Pan, 2001). The most parsimonious model was selected with the quasi-likelihood framework or quasi-AIC which estimates the quality of each model relative to other models and minimises the amount of information loss. The response variables for the full model were chosen with a stepwise approach with forward entry from the null model with a modified model selection criterion (Carvalho, 2010) (Table 4).

Table 4. The stepwise approach to model selection with quasi-AIC.

Run	Model
1	<i>CPUE = YEAR</i>
2	<i>CPUE = YEAR + SEASON</i>
3	<i>CPUE = YEAR + SEASON + SST</i>
4	<i>CPUE = YEAR + SEASON +SST +TACTIC</i>
5	<i>CPUE = YEAR + SEASON + SST + TACTIC + LOA</i>
6	<i>CPUE = YEAR + SEASON + SST +T ACTIC + LOA + LAT</i>
7	<i>CPUE = YEAR + SEASON + SST + TACTIC + LOA + LAT + LONG</i>
8	<i>CPUE = YEAR + SEASON + SST + TACTIC + LOA + LAT + LONG + (LOA*LAT)</i>
9	<i>CPUE = YEAR + SEASON + SST + TACTIC + LOA + LAT + LONG + (LOA*LAT)+ (LOA*LONG)</i>
10	<i>CPUE = YEAR + SEASON + SST + TACTIC + LOA + LAT + LONG + (LOA*LAT) + (LOA*LONG) + (SEASON*SST)</i>

Residual deviance explained by each explanatory variable was determined by sequentially comparing smaller models with more complex models with likelihood ratio tests (LRT). Deviance is a measure of how much the fitted values differ from the observations. The distribution trend of the standardised residual deviance plotted with the fitted values was a diagnostic tool to evaluate the model fit. Additionally, plots of the frequency of standardised residual deviance and quantile-

quantile (Q-Q) plots were checked for normal distribution of standardised residual deviance. The Q-Q plot compared the standardised residual deviance of the model with that of theoretical quantiles.

## Results

### *Fisheries catch and effort data*

The peaks in the number of swordfish caught in 2002 and 2011 are correlated with peaks in the number of hooks deployed in the East ( $\geq 27^\circ\text{E}$ ) (Figure 4). In the West ( $\leq 15^\circ\text{E}$ ) however, the number of swordfish caught has been declining since 2001, despite a consistent level of effort (Figure 4).

The frequency of nominal CPUE values from 1998 to 2014 for the West ( $\leq 15^\circ\text{E}$ ) and East ( $\geq 27^\circ\text{E}$ ) indicates the distribution of CPUE data and the extent of zero-catch data (Figure 5 (a) and (b)). The average percentage of zero catch is 15.1% and 16.3% for the West ( $\leq 15^\circ\text{E}$ ) and East ( $\geq 27^\circ\text{E}$ ), respectively, with increasing proportions of zero catch over the time period (Figure 6).

### *Clustering*

The *k*-means clustering algorithm in Cluster 3.0 indicated 2 clusters in the West ( $< 20^\circ\text{E}$ ) area, 3 clusters in the West ( $\leq 15^\circ\text{E}$ ) area, 2 clusters in the East ( $\geq 20^\circ\text{E}$ ) area and 2 clusters in East ( $\geq 27^\circ\text{E}$ ) area. Detailed results of the clustering are provided for the two main models, West ( $\leq 15^\circ\text{E}$ ) and East ( $\geq 27^\circ\text{E}$ ). The three clusters in the West can be grouped into a target species or group of species according to percentage number of each species caught: Cluster 1) blue shark (92.3%); Cluster 2) albacore (70.5%); and Cluster 3) albacore (25.3%), bigeye tuna (19.4%), swordfish (19.7%) and blue shark (15.9%) (Table 3a). Similarly, the two clusters in the East can be grouped into a target species or group of species according to percentage number of each species caught: Cluster 1) bigeye tuna (22.7), swordfish (20.5%), yellowfin tuna (16.1%), albacore (15.7%); Cluster 2) yellowfin tuna (76.2%) (Table 3b). Cluster 3 and Cluster 1, both dominated by a combination of tunas, swordfish and blue shark targeted, has been the majority fishing tactics over the time scale in the West ( $\leq 15^\circ\text{E}$ ) and East ( $\geq 27^\circ\text{E}$ ), respectively (Figure 7). In the West ( $\leq 15^\circ\text{E}$ ), the percentage of Cluster 1 (i.e. blue shark dominated) increased in 2011 and 2012 (Figure 8).

### *Power parameter*

The value of  $p$  corresponding to Maximum Likelihood Estimates (MLE) for a Poisson distribution was approximately 1.44 and 1.46 for the West ( $\leq 15^\circ\text{E}$ ) and East ( $\geq 27^\circ\text{E}$ ), respectively (Figure 9).

#### *Model procedure*

The final model for the West ( $\leq 15^\circ\text{E}$ ), West ( $< 20^\circ\text{E}$ ), East ( $\geq 27^\circ\text{E}$ ) and East ( $\geq 20^\circ\text{E}$ ) with the lowest AIC score was:

$$CPUE = \text{Intercept} + \text{Year}_i + \text{Season}_j + \text{SST}_k + \text{LOA}_l + \text{Tactic}_m + \text{LAT}_n + \text{LONG}_o + (\text{LOA} * \text{LAT})_{ln} + (\text{LOA} * \text{LONG})_{lo} + (\text{SEASON} * \text{SST})_{jk} + \text{offset}(\text{Hook}) + \text{Error}_{ijklmn} \quad (11)$$

#### *Summary statistics*

The final model explained 49.21% of the variance in the CPUE in the West ( $\leq 15^\circ\text{E}$ ), and 61.95% of the variance in the East ( $\geq 27^\circ\text{E}$ ) (Table 4). The Year (39.99%) and Season (27.17%) factors explained a majority of the variance in the CPUE in the West ( $\leq 15^\circ\text{E}$ ). The latitude explains 8.77% of the variation, which is evident in the map of the nominal CPUE that depicts a hotspot of swordfish further north (Figure 1). The tactic was not a significant variable in the model, explaining only 0.18% of the variation. The LOA (41.85%) and Year (17.49%) factors explained a majority of the variance in the CPUE in the East ( $\geq 27^\circ\text{E}$ ). The LOA\*LAT interaction term (9.64%) indicates a separation of vessel size by latitude. The smaller South African-flagged vessels in this area operate out of Richards Bay in the higher latitudes, whereas the foreign-flagged vessels operate in the lower latitudes of this region targeting tuna. The histograms of standardised residual deviances appear normal (Figure 10). The quantile-quantile (Q-Q) plot skewed slightly on the right hand side in both models (Figure 11).

#### *Standardised CPUE*

The relative abundance in the South Atlantic Ocean has been on a steady declining trend throughout the time frame of this fishery in the West ( $\leq 15^\circ\text{E}$ ) and West ( $< 20^\circ\text{E}$ ) models (Figure 12 (a)). In the Indian Ocean, the East ( $\geq 27^\circ\text{E}$ ) and East ( $\geq 20^\circ\text{E}$ ) models indicate that relative abundance appears to have stabilised from 2004 to 2011 before declining from 2011 onwards (Figure 12 (b)). The trends of the models that included the admixture zone, West ( $< 20^\circ\text{E}$ ) and East ( $\geq 20^\circ\text{E}$ ), were the same as their counterpart models that excluded the admixture zone, West ( $\leq 15^\circ\text{E}$ ) and ( $\geq 27^\circ\text{E}$ ), respectively.

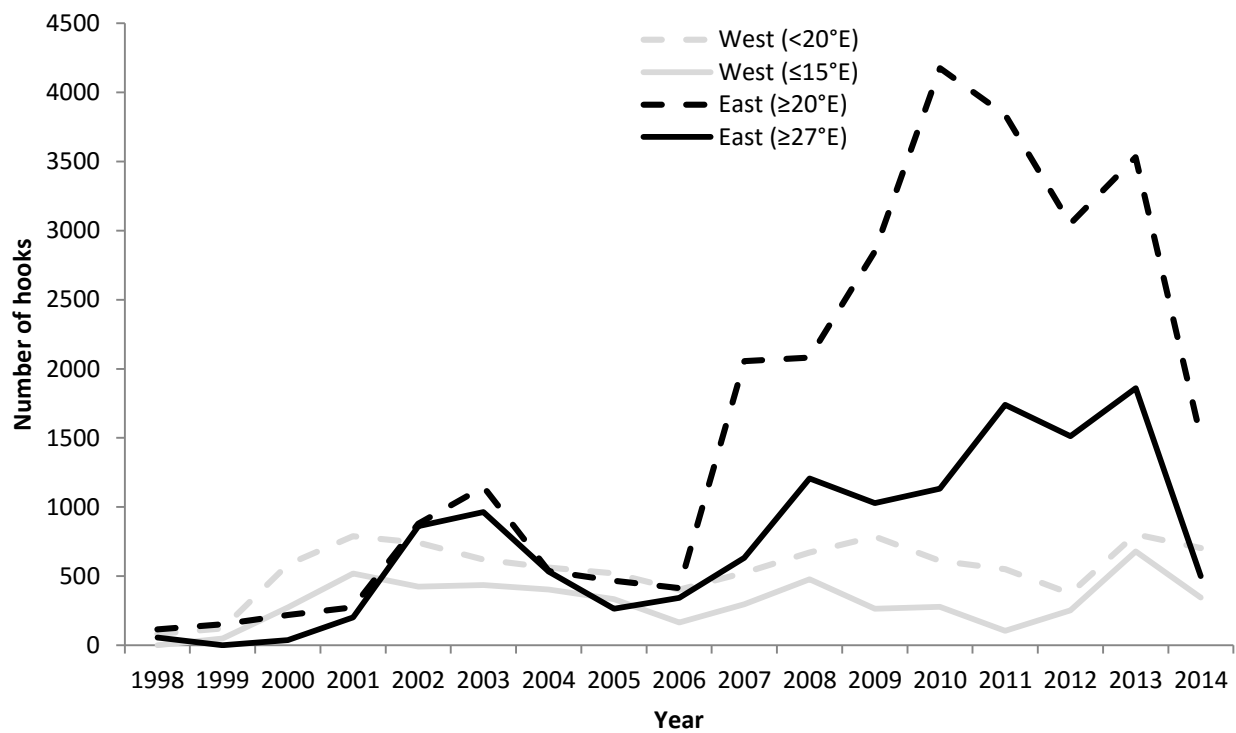
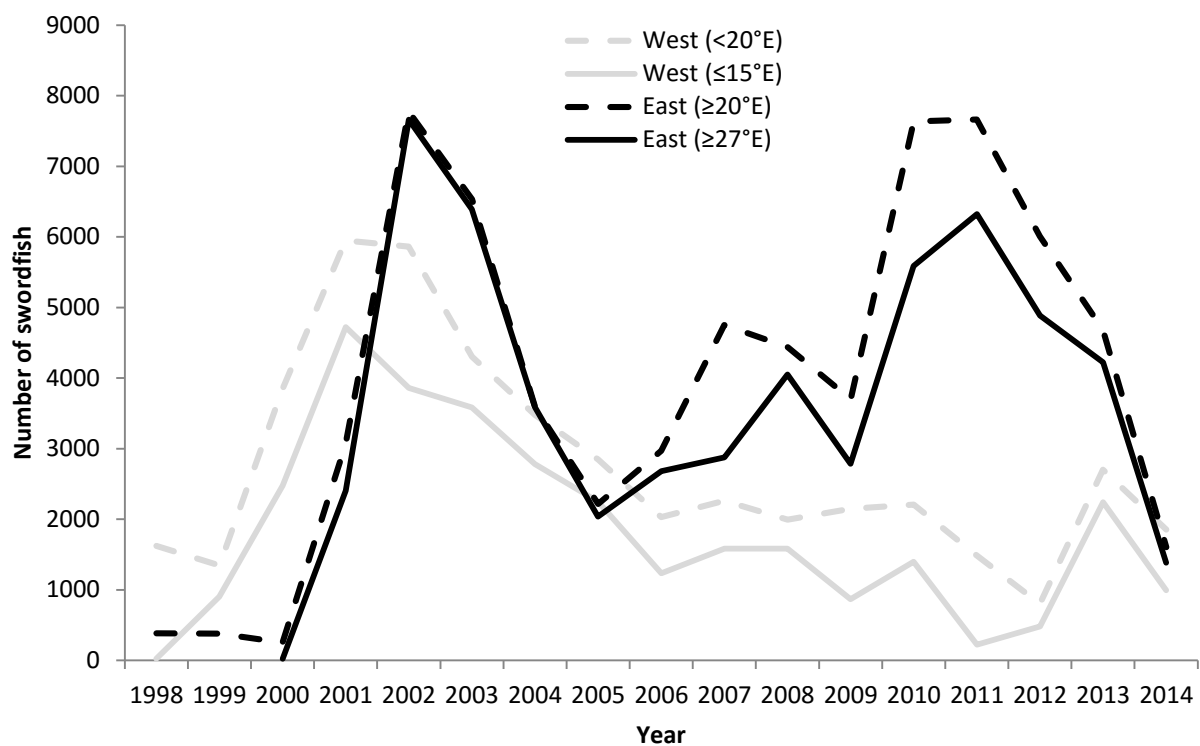


Figure 4. Swordfish catch (in numbers) and total effort (number of hooks) after applying filtering criteria for the West ( $\leq 15^{\circ}\text{E}$ ), West ( $< 20^{\circ}\text{E}$ ), East ( $\geq 27^{\circ}\text{E}$ ) and East ( $\geq 20^{\circ}\text{E}$ ) in the GLM analysis.

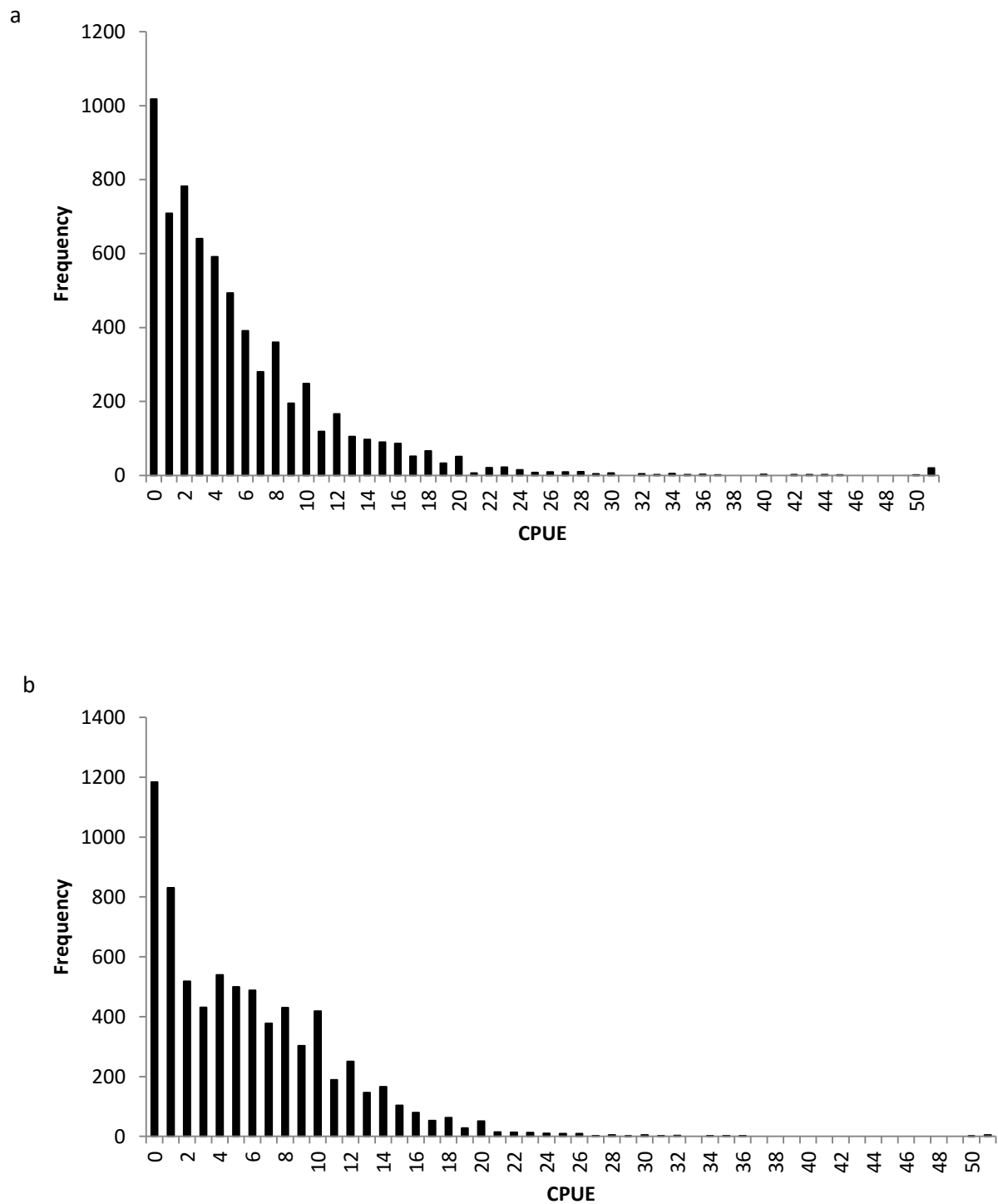


Figure 5. Frequency of nominal CPUE values for longline sets made from 1998 to 2014 in (a) the West ( $\leq 15^\circ\text{E}$ ) and (b) the East ( $\geq 27^\circ\text{E}$ ).



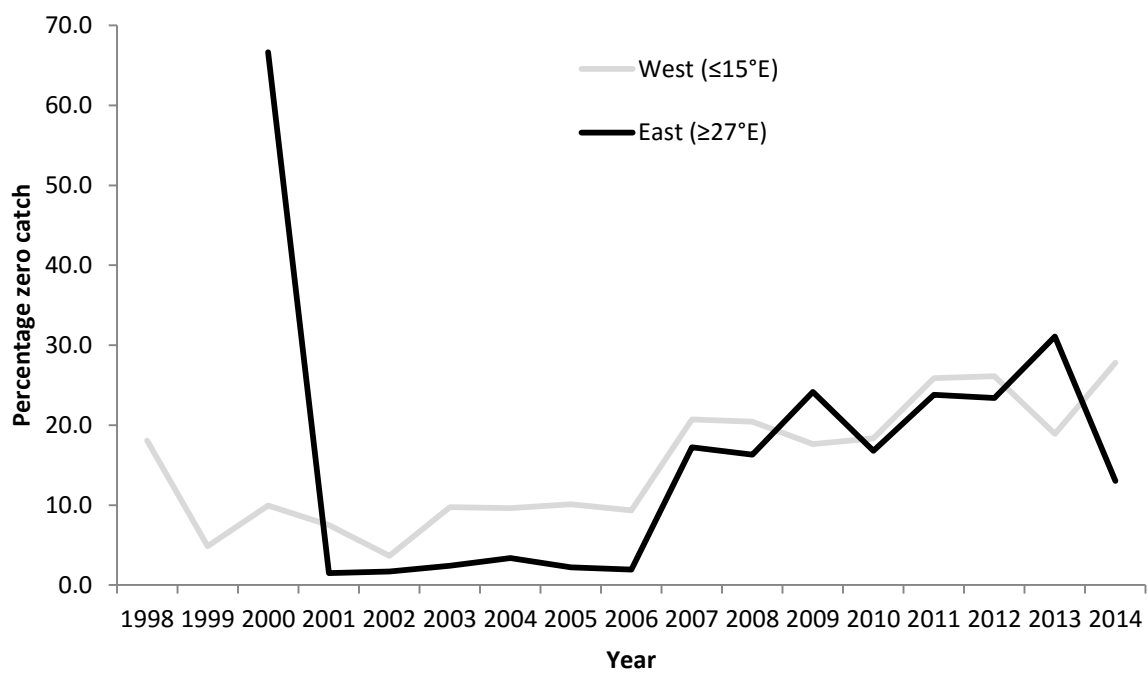


Figure 6. The percentage of sets per year with zero swordfish catch for longline sets made from 1998 to 2014 for the West ( $\leq 15^{\circ}\text{E}$ ) and East ( $\geq 27^{\circ}\text{E}$ ).

Table 3. Percentage of species (in number) caught per targeting strategy cluster in (a) the West ( $\leq 15^{\circ}\text{E}$ ) and (b) East ( $\geq 27^{\circ}\text{E}$ ).

a

Species	Cluster 1	Cluster 2	Cluster 3
Bigeye tuna	0.6	8.2	<b>19.4</b>
Yellowfin tuna	0.1	3.1	2.0
Swordfish	0.8	8.0	<b>19.7</b>
Mako shark	2.0	2.4	5.7
Blue shark	<b>92.3</b>	4.0	<b>15.9</b>
Albacore	2.6	<b>70.5</b>	<b>25.3</b>
Southern bluefin tuna	0.0	0.4	1.1
Unidentified tuna	0.0	0.0	0.7
Black marlin	0.0	0.0	0.1
Blue marlin	0.0	0.0	0.0
Unidentified marlin	0.0	0.0	0.1
Sailfish	0.0	0.0	0.0
Unidentified shark	0.0	0.1	1.1
Other fish	0.0	0.0	0.2
Dorado	0.0	0.1	0.1
Oilfish	1.4	2.8	8.2
Escolar	0.0	0.2	0.4
Wahoo	0.0	0.0	0.0
Moon fish	0.0	0.2	0.1

b

Species	Cluster 1	Cluster 2
Bigeye tuna	<b>22.7</b>	9.5
Yellowfin tuna	<b>16.1</b>	<b>76.2</b>
Swordfish	<b>20.5</b>	1.7
Mako shark	1.1	0.6
Blue shark	5.2	2.1
Albacore	<b>15.7</b>	4.1
Southern bluefin tuna	0.6	0.1
Unidentified tuna	1.4	0.3
Black marlin	0.3	0.1
Blue marlin	0.2	0.1
Unidentified marlin	1.0	0.1
Sailfish	0.2	0.0
Unidentified shark	0.4	0.0
Other fish	1.7	0.0
Dorado	4.8	0.5
Oilfish	4.7	0.7
Escolar	2.2	3.3
Wahoo	0.1	0.0
Moon fish	1.3	0.5

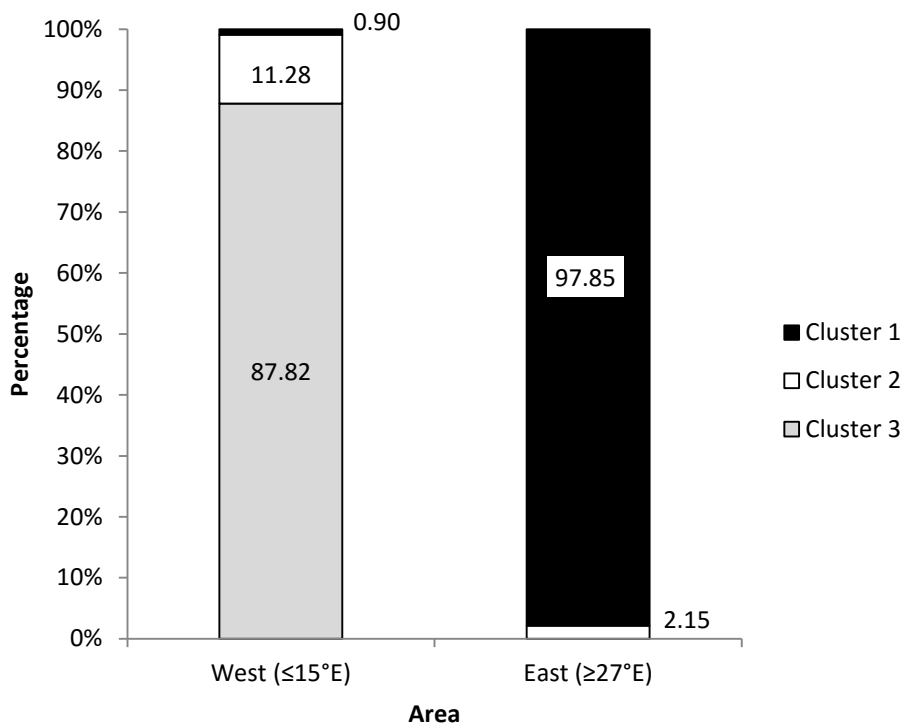


Figure 7. The average percentage of the number of longline sets classified as cluster 1, 2 and 3 in the West ( $\leq 15^\circ\text{E}$ ) and East ( $\geq 27^\circ\text{E}$ ) from 1998 to 2014.

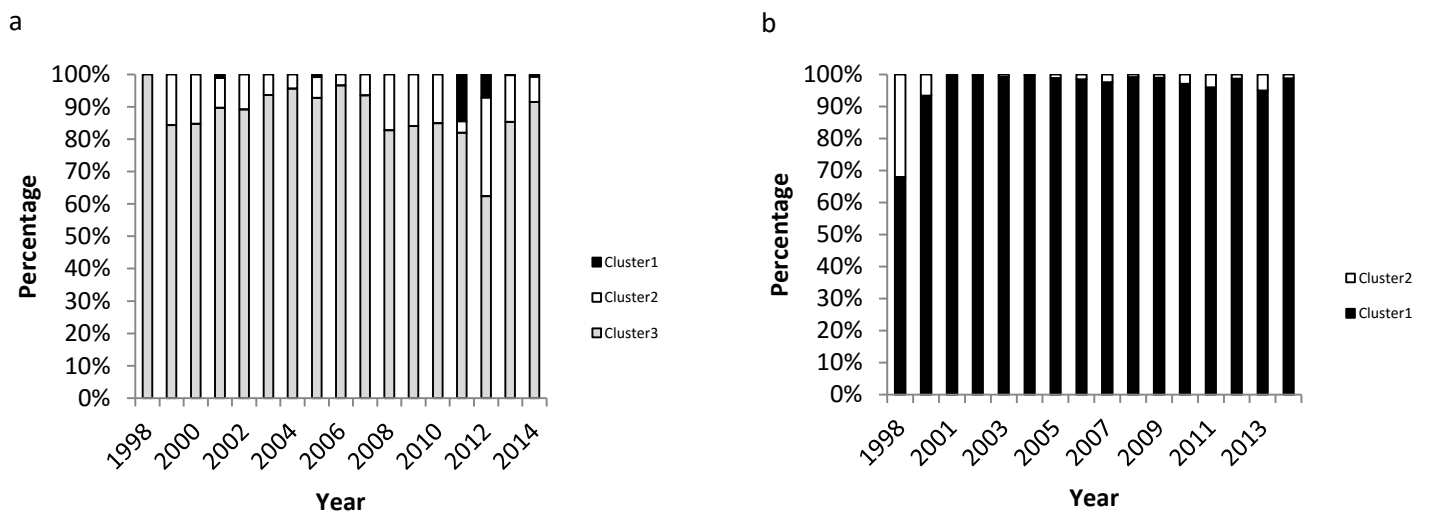
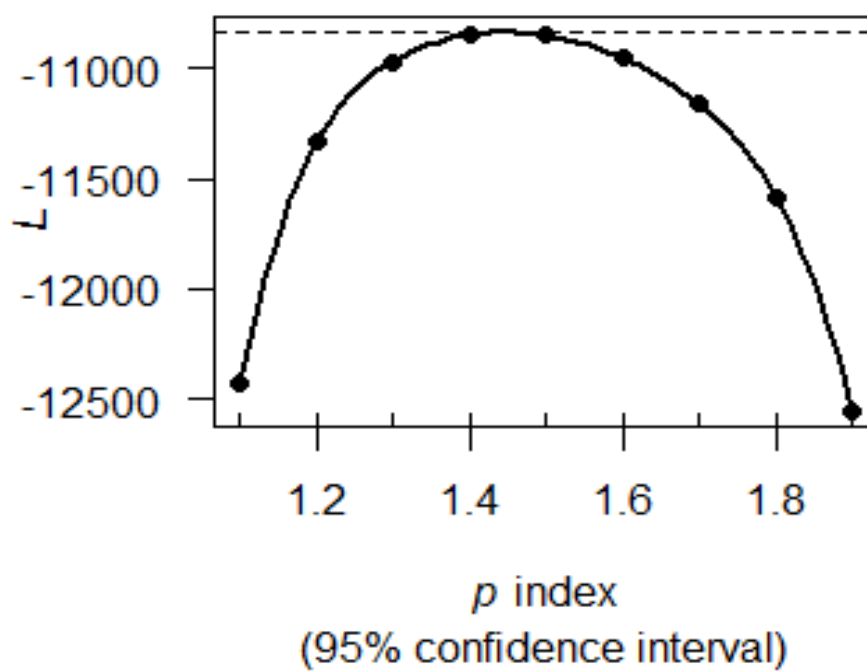


Figure 8. The targeting strategy or tactic for each year in (a) the West ( $\leq 15^\circ\text{E}$ ) and (b) the East ( $\geq 27^\circ\text{E}$ ).

a



b

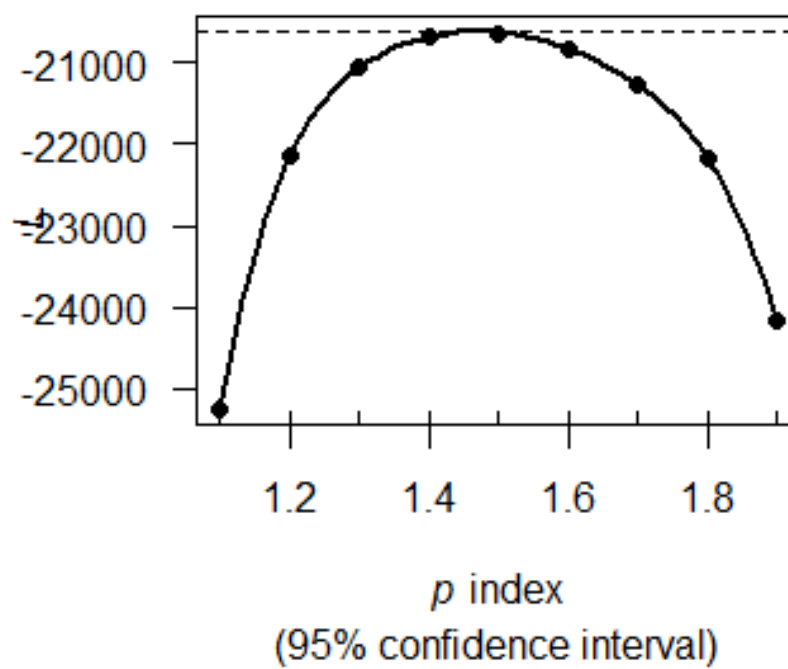


Figure 9. The value of  $p$  corresponds to the MLE (maximum likelihood estimate) in (a) the West ( $\leq 15^\circ\text{E}$ ), and (b) the East ( $\geq 27^\circ\text{E}$ ).

Table 4. Summary statistics for (a) the West ( $\leq 15^\circ\text{E}$ ) and (b) the East ( $\geq 27^\circ\text{E}$ ). ‘\*\*\*’ $p < 0.0$ , ‘\*\*’ $p < 0.001$ , ‘.’ not significant. Degrees of freedom (d.f.), Residual (Res.), Deviance (Dev.), Akaike information criterion (AIC), Delta ( $\Delta$ ).

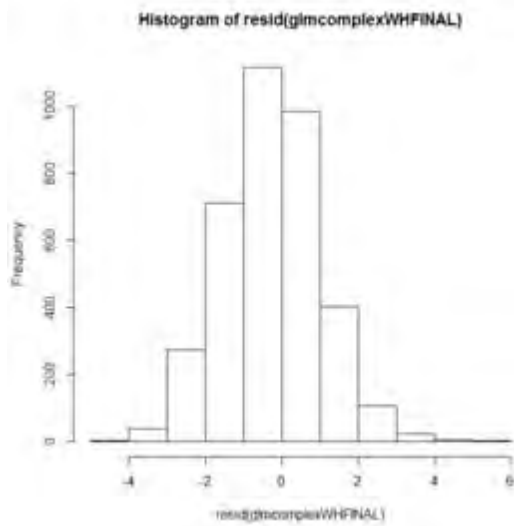
Parameter	d.f.	Res. d.f.	AIC	$\Delta$ AIC	Deviance	Res. Dev.	$\Delta$ Dev	% explained deviance	% explained model	$p$
NULL		6729	21837.27			13466.4				
YEAR	16	6713	20940.09	-897.18	2650.02	10816.4	-2650	39.99	19.68	***
SEASON	3	6710	20101.2	-838.89	1800.43	9016	-1800	27.17	33.05	***
SST	5	6704	19898.67	-202.53	496.05	8519.9	-496	7.49	36.73	***
TACTIC	2	6703	19896.17	-2.5	11.61	8508.3	-12	0.18	36.82	.
LOA	2	6701	19851.5	-44.67	285.65	8222.6	-286	4.31	38.94	***
LAT	27	6673	19629.22	-222.28	581.27	7641.4	-581	8.77	43.26	***
LONG	15	6654	19430.29	-198.93	330.01	7311.4	-330	4.98	45.71	***
LOA:LAT	27	6622	19385.16	-45.13	200.55	7110.8	-201	3.03	47.20	***
LOA:LONG	30	6584	19343.27	-41.89	207.66	6903.2	-208	3.13	48.74	***
SEASON:SST	10	6469	19078.7	-264.57	63.81	6839.4	-64	0.96	49.21	***

% deviance explained **49.21**

Parameter	d.f.	Res. d.f.	AIC	$\Delta$ AIC	Deviance	Res. Dev.	$\Delta$ Dev	% explained deviance	% explained model	$p$
NULL		7251	41582.16			32304				
YEAR	15	7236	40572.89	-1009.27	3500	28804	-3500	17.49	10.83	***
SEASON	3	7233	40079.7	-493.19	1433.7	27371	-1433	7.16	15.27	***
SST	6	7227	39543.77	-535.93	1773.3	25597	-1774	8.86	20.76	***
TACTIC	1	7226	39386.63	-157.14	542.3	25055	-542	2.71	22.44	***
LOA	2	7224	63576.62	24189.99	8374.7	16680	-8375	41.85	48.37	***
LAT	18	7206	36447.5	-27129.12	513.1	16167	-513	2.56	49.95	***
LONG	17	7189	36095.33	-352.17	854.4	15313	-854	4.27	52.60	***
LOA:LAT	25	7164	35504.89	-590.44	1928.7	13384	-1929	9.64	58.57	***
LOA:LONG	28	7136	35280.48	-224.41	1018.9	12365	-1019	5.09	61.72	***
SEASON:SST	9	7127	35267.86	-12.62	74	12291	-74	0.37	61.95	**

% deviance explained **61.95**

a



b

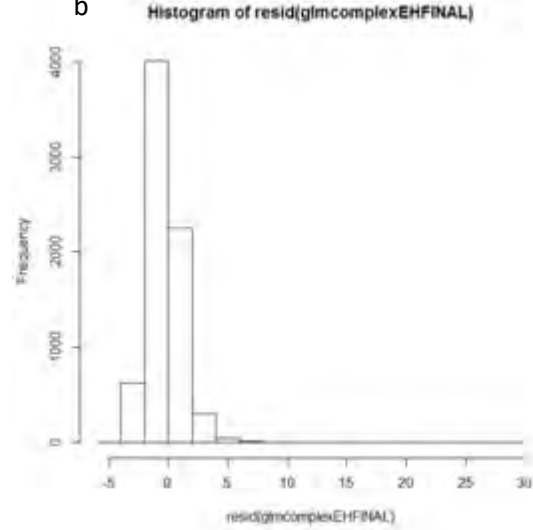
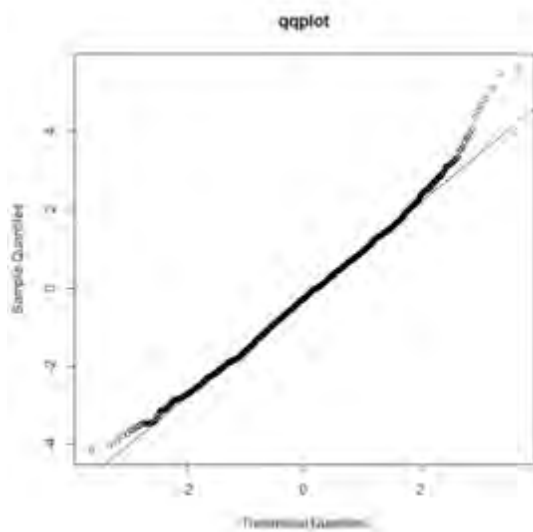


Figure 10. Histogram of standard deviance residuals for (a) the West ( $\leq 15^\circ\text{E}$ ) and (b) the East ( $\geq 27^\circ\text{E}$ ).

a



b

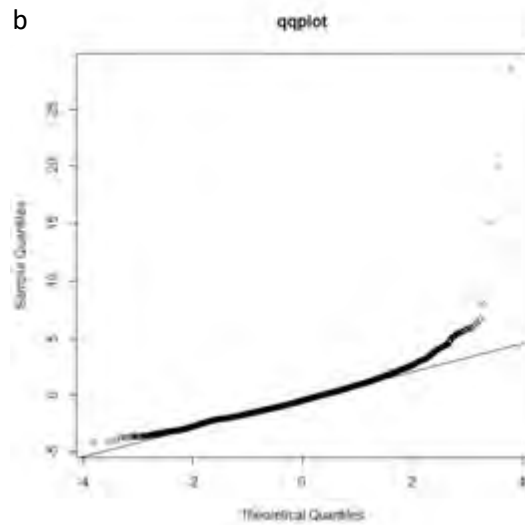


Figure 11. Quantile-quantile (Q-Q) plots of the deviance residuals for (a) the West ( $\leq 15^\circ\text{E}$ ) and (b) the East ( $\geq 27^\circ\text{E}$ ).

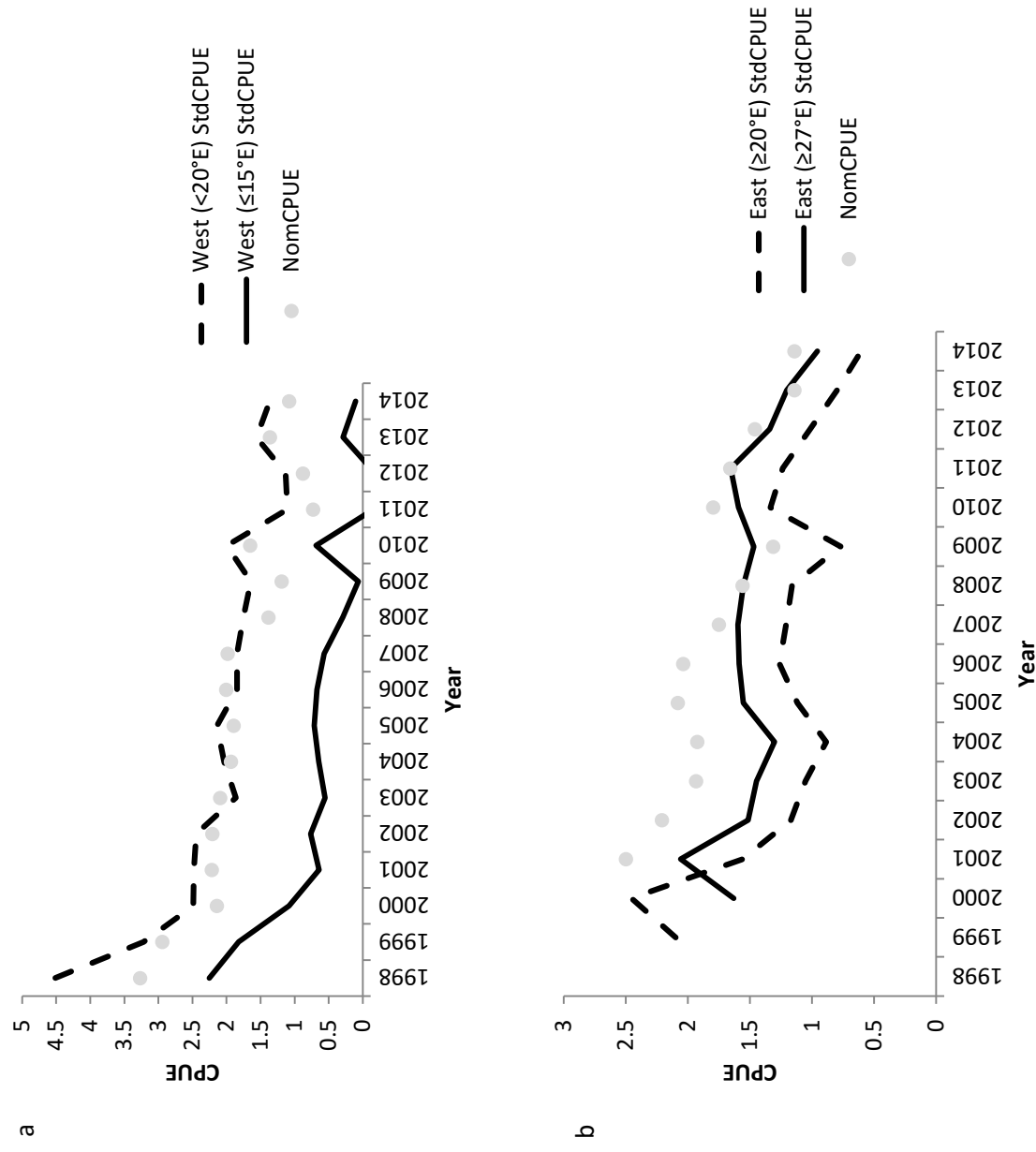


Figure 12. Year trends of standardised and nominal CPUE for swordfish caught by the South African pelagic longline fleet from 1998 to 2014 in (a) the West ( $\leq 15^{\circ}\text{E}$ ) (solid) and West ( $< 20^{\circ}\text{E}$ ) (stippled line), and (b) the East ( $\geq 20^{\circ}\text{E}$ ) (solid) and East ( $\geq 27^{\circ}\text{E}$ ) (stippled). The nominal CPUE for (a) West ( $\leq 15^{\circ}\text{E}$ ) and (b) East ( $\geq 27^{\circ}\text{E}$ ) is indicated by the grey points.

## Discussion

### *Abundance trends*

In the West the effort has been concentrated between the 15°S to 30°S latitude (Figure 2). However, the swordfish nominal CPUE indicates that areas further south in the 30°S to 40°S latitude are more suitable for improved catch performance of swordfish (Figure 1). In the East effort has been concentrated in the 20°E to 35°E longitude, though swordfish nominal CPUE is highest further north in the 35°E – 45°E region (Figure 1 and 2), a potential hotspot for swordfish. The dissimilarity in the areas of effort and nominal CPUE is likely due to the inclusion of catch data from foreign-flagged vessels that generally target tuna species. The South African vessels store their catch on ice, and this limits their trips to average two week duration and in relatively close proximity to the port of call, Cape Town or Richards Bay (Figure 1).

The standardised CPUE peaked at the beginning of the fishery in 1999 and from 2002 to 2003 in the West and East, respectively. At the start of the fishery from 1997 until 2002, monitoring of catches during offloading in port and the placement of observers on local and foreign longline vessels was not yet well established. There has very likely been underreporting of catches during this period due to quota limitations and bycatch restrictions. The standardised CPUE may have reflected a higher maximum and steeper decline in abundance after 2003, were these catches reported.

The standardised CPUE in the West experienced a small spike in 2010. The 2011-2012 drop coincides with increased Cluster 1 (i.e. blue shark) targeting strategy (Figure 8). Since the tactic factor accounts for only 0.18% of the variation in the model, it is unlikely that the pattern is an artefact of the change in targeting. Though there was a peak in 2013, the abundance continues to decline (Figure 12 (a)).

The declining abundance of swordfish in the Atlantic (West) is in agreement with some and disagreement of other studies from fleets that fish in the Atlantic. The studies by Ortiz (2007) (U.S.A.), Pons *et al.* (2014) (Uruguay) and Chang *et al.* (2007) (Taiwan) indicate a declining abundance trend. The studies by Hazin *et al.* (2014) (Brazil) and Santos *et al.* (2014a) (Portugal) indicate increasing trends in swordfish abundance. Fleets with fluctuating and stable trends include examples such as Sun *et al.* (2014) (Taiwan), Mourato *et al.* (2007) (Santos, Brazil) and Ramos-Cartelle *et al.* (2014) (Spain). The study by Yokawa and Kai (2014) for the Japanese longline fleet indicates a declining trend followed by an increase in abundance. The catch and effort in the Indian (East) experienced a peak from 2010 to 2013 (Figure 4), yet the standardised CPUE has been stable



from 2005 to 2011 and declining thereafter (Figure 12 (b)), an indication that although the fishery was successfully catching larger numbers of swordfish, this was not due to a higher abundance of swordfish. Both dominant targeting clusters/tactics include swordfish and the percentage of each cluster has not experienced visibly significant changes in recent years (Figure 9b). The abundance trend by Nishida and Wang (2014) (Japan) also indicates a declining abundance trend. However, the studies by Santos *et al.* (2014b) (Portugal, South West Indian Ocean) and Santos *et al.* (2013) (Portugal, Indian Ocean) have been stable and the study by Wang and Nishida (2014) (Taiwan) has been fluctuating yet stable. It is important to note that scopes of each of these studies that have been listed differ with respect to their fisheries, the oceanographic conditions, area and subpopulations tracked and comparisons have limitations.

#### *Model parameters*

The tweedie model was able to explain a high percentage of variation. The importance of the Year factor in explaining variation could be attributed to the age of this fishery. As the fishery develops in facets such as gear, skills, size, market for product etc. the fishery will have annual variation before it reaches stability.

Change in regional environmental and/or oceanographic conditions cannot be ruled out as an explanation for the decrease in abundance (*e.g.* Podesta *et al.*, 1993; Bigelow *et al.*, 1999, Damalas *et al.*, 2007). Nishida *et al.* (2011) utilised anomalies of environmental data (*e.g.* Indian Ocean Index, Indian Ocean Dipole Index, shear current amplitudes of the shear current) as suggested by Kolody *et al.* (2010) and Nishida and Wang (2010). However none of these factors tested significant in the GLM analyses and few environmental factors (moon phase, thermocline depth and temperature gradient at 45 m depth) suggested significant effects. The inclusion of environmental and/or oceanographic conditions should be considered in the future.

The targeting strategies may change in coming years if vessels continue to experience poor swordfish catch performance and the vessels remain in the near shore fishing grounds. South Africa's recent (September, 2015) accession to the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) will increase the country's southern bluefin quota from 40t to 150t for 2016 to 2017 and there is potential for higher allocations in the future. Vessels might direct their effort towards this lucrative resource in the future. On the other hand, the DAFF 2015/2016 Large Pelagic

Longline Policy has prohibited the use of wire traces on all pelagic longline vessels to reduce the pelagic shark directed catch and effort. This restriction could result in increased targeting of tunas and billfish species.

Scores from Principal Component Analysis (PCA) has been suggested as a more effective indicator of targeting than the cluster analysis approach (*e.g.* Ortega-García and Gómez-Muñoz, 1992; Pech and Laloë, 1997; MacNeil *et al.*, 2009; Winker *et al.*, 2013; Hoyle *et al.*, 2014; Winker *et al.*, 2014). In Wang (2015) the PCA was performed based on the linear regression models constructed of the catch compositions of six main species groups. The principal component scores, derived from the PCA of the catch composition data, were used as continuous nonlinear predictor variables for targeted effects in the CPUE standardisation model. This approach to account for targeting in multispecies CPUE can be considered in future model runs.

Kolody (2011) provided a concise summary of the problems in the standardised longline CPUEs of the southwest Indian Ocean sub-population; problems that are not unique to this area but have application for all species, stocks and gear types that use standardised CPUE as indicators of abundance. The challenges of this method includes: the selection of appropriate spatial units, differences in trends of fleets that operate in the same area, the strong influence that individual fleets may have on the outcome, changes in spatial distribution of effort, changes in gear and gear deployment, changes in targeting, understanding environmental influences and the selection of the most suitable model. These challenges are ongoing and require that future studies remain mindful of the nuances when interpreting standardised CPUE results.

#### *Localised depletion*

Stock assessments have been conducted separately for the SWIO region by the Indian Ocean Tuna Commission (IOTC) over concerns that this area may have a sub-population or stock of swordfish that is undergoing localised depletion. South Africa's abundance index in the East, i.e. within the SWIO region, matches the declining trend displayed by the Portuguese (*e.g.* Santos *et al.*, 2013; Santos *et al.*, 2014b). The increasing trend of sets with zero swordfish catch reflects the poor catch performance.

The South African swordfish abundance has been on a steady declining trend since its peak around 2000, and a contributing factor could be localised depletion of the resource. Swordfish are oceanic

species but are known to occur in coastal waters (Collette, 1995). Through satellite tagging studies, there is evidence of swordfish displaying homing behaviour to temperate foraging areas (*e.g.* Nielsen *et al.*, 2009; Nielsen and Smith, 2010) and will return annually to, and display fidelity towards, specific feeding areas (Sperling *et al.*, 2005; Kolody *et al.*, 2006; Holdsworth *et al.*, 2010). Swordfish tagged in the North Atlantic migrated south towards a known spawning area in the Caribbean Sea before the northward migration to temperate waters resumes (Nielsen *et al.*, 2009). It has been suggested that localised sub-populations that will remain in particular locations driven by environmental conditions (Muths *et al.*, 2013, in the south west Indian Ocean) that influences food availability (*e.g.* offshore seamounts, submarine canyons, thermal fronts) (Podestá *et al.*, 1993; Sedberry and Loefer, 2001; Campbell and Hobday, 2003). The older, mature individuals will migrate seasonally to the spawning grounds and return to productive feeding areas thereafter (Poisson, 2009). Swordfish that come back to the sub-population “pockets” are vulnerable to overfishing due to this semi-residency. If swordfish have a replenishment rate that is slower than the rate they are being removed, then the sub-populations will decline. The high catch rates and abundance followed by a decline for South Africa has also been witnessed at the ‘Brisbane grounds’ in Australia’s dedicated swordfish fishery after 1997 (Campbell, 2002; Campbell and Hobday, 2003). In Australia, the fleet has experienced repeated patterns of “localised decline” as the fleet expands operations into new areas further offshore.

Fleets that have limited ability to search for fish further offshore, and that are swordfish-directed, are sensitive to and vulnerable to changes in swordfish abundance. A regional (South Atlantic stock, Indian Ocean stock) stock assessment could indicate a degree of hyper-stability for the core region whilst the abundance declines in the peripheral areas. South Africa is in the southern range limit for swordfish (Itano, 2011) and the vessels have remained in the coastal regions (*i.e.* within and around the 200 nm EEZ), limited by their vessel type (*e.g.* ice vessels) and vessel size. Re-population of an area that is in the furthest limit range for that species in the presence of fishing pressure may only be achieved in the long term. If swordfish display a relatively high degree of affinity to features such as seamounts, then as the sub-populations on these features are fished down, it would appear that they are not readily replenished by swordfish that associate with other features in the region (Campbell, 2002). This implies a high ‘stickiness’ or viscosity of the swordfish resource, *i.e.* the movement of fish away from a resident area into an area heavily fished is relatively slow (Campbell, 2002).

The big old fat fecund female fish (BOFFFF) hypothesis proposed by Berkley *et al.* (2004) implies that older females produce more and better quality offspring as they age. Poisson and Fauvel (2009) highlighted the important role of the older/larger females in the reproductive capacity of south western Indian Ocean stock and the potential implications of fishing the older/larger females for this stock in terms of reproduction and recruitment and recovery of the stock.

#### *Admixture zone*

In the area around South Africa where the Atlantic Ocean and Indian Ocean meets, large pelagic species that are recognised as two stocks from within each Ocean interact in various different ways. Each species has a unique behavioural pattern in this area and its stock dynamics should be studied individually. The potential admixture zone, as revealed by this genetics study, can be scrutinised further to have a clearer understanding of the dynamics of swordfish at this junction. Subsequently, scientifically motivated management decisions need to guide how the catch data is analysed in this region. In this study, the genetically determined admixture zone was included, and excluded, from the models. The trends of the models that had admixed data (West  $<20^{\circ}\text{E}$  and East  $\geq 20^{\circ}\text{E}$ ) were not visually different from the models that excluded this data (West  $\leq 15^{\circ}\text{E}$  and East  $\geq 27^{\circ}\text{E}$ ) (Figure 12). This analysis reveals that whether the tuna RFMOs recognise the admixed area around South Africa or not, the abundance index from South Africa can be included at the current management boundary at  $20^{\circ}\text{E}$ . These analyses can be furthered with the data from fleets such as the Japanese and Taiwanese which fish further offshore than the area around South African have larger volumes of historical data.

Although the abundance trends of the admixed and non-admixed models are similar, scientists and managers would still need to consider how the tonnages of catch in this area is divided between the Indian and Atlantic Ocean stocks when estimating fishing mortality.

In conclusion, the South African fleet's swordfish abundance trends are in accordance with fleets operating in the same area, such as the SWIO, or from coastal regions like Brazil and Uruguay. Since the latitudinal and longitudinal range of the South African fleet is protracted in relation to the entire South Atlantic and Indian Ocean regions, the declining trends may be indicative of localised hyper-depletion in these areas, as experienced in Australia and Reunion Island (Govender *et al.*, 2003), and not an accurate reflection of the state of the two stocks. If the vessels continue to direct effort towards swordfish in the same areas, the abundance trend will not change its trajectory and the

overall viability of this fishery has to be questioned. Improvements to the study through the inclusion of environmental data and different techniques to address targeting, can be implemented in future analyses. The dynamics of the South African fleet may change in coming years as targeting changes, and collecting data that is relevant and reliable will be important for future estimations of abundance.

## References

- Akaike, H. (1973). Information theory as an extension of the maximum likelihood principle. In: Petrov, B. N. and Csaki, F., eds., *Second International Symposium on Information Theory*. Akademiai Kiado, Budapest, pp. 267 – 281.
- Anderson, D., Feldblum, S., Modlin, C., Schirmacher, D., Macher, E. and Thandi, N. (2004). A practitioner's guide to generalized linear models. *Casualty Actuarial Society. Discussion Papers*, pp. 1 – 116.
- Andrews, W. R. H. and Hutchings, L. (1980). Upwelling in the Southern Benguela current. *Progress in Oceanography*, 9, pp. 1.
- Berkeley, S.A., Hixon, M.A., Larson, R.J. and Love, M.S. (2004). Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries*, 29, pp. 23 – 32.
- Beverton, R.J.H. and Parrish, B.B. (1956). Commercial statistics in fish population studies. In: *Problems and methods of sampling fish populations*. International Council of the Exploration of the Sea, 140, pp. 58 – 66.
- Beverton, R.J.H. and Holt, S.J. (1957). *On the dynamics of exploited fish populations*. Fishery Investigations, London, Series II (XIX), 533pp.
- Bigelow, K.A., Boggs, C.H. and He, X. (1999). Environmental effects on swordfish and blue shark catch rates in the US North Pacific longline fishery. *Fisheries Oceanography*, 8, pp. 178–198.
- Bigelow, K. A., Langley, A. D. and Patterson, T. (2004). Relative abundance indices of the Japanese longline fishery for bigeye and yellowfin tuna in the western and central Pacific Ocean. WP SA-7, SCTB 17:9-18.
- Brodziak, J. and Walsh, W.A. (2013). Standardizing catch rates of bycatch species using multimodel inference: A case study of oceanic whitetip shark in the Hawaii longline fishery. *Canadian Journal of Fisheries and Aquatic Sciences*, 70, pp. 1723 – 1740.
- Campbell, R. (2002). Sequential changes in swordfish catch rates off eastern Australia and possible implications for the spatial distribution of the local swordfish population [BBRG-9]. Noumea, New

- Caledonia: SPC, Secretariat of the Pacific Community. Standing Committee on Tuna and Billfish, Honolulu, Hawaii, U.S.A., 22-27 July 2002, 13 p.
- Campbell, R. and Hobday, A. (2003). Swordfish - seamount - environment - fishery interactions off Eastern Australia [BBRG-3]. Noumea, New Caledonia: SPC, Secretariat of the Pacific Community. Standing Committee on Tuna and Billfish, Mooloolaba, Queensland, Australia, 9-16 July 2003, 23 p.
- Campbell, R. A. (2004). CPUE standardisation and the construction of indices of stock abundance in a spatially varying fishery using general linear models. *Fisheries Research*, 70, pp. 209 – 227.
- Campbell, R. A. (2015). Constructing stock abundance indices from catch and effort data: Some nuts and bolts. *Fisheries Research*, 161, pp. 109 – 130.
- Cao, J., Chen, X., Chen, Y., Liu, B., Ma, J. and Li, S. (2011). Generalised linear Bayesian models for standardising CPUE: an application to a squid-jigging fishery in the northwest Pacific Ocean. *Scientia Marina*, 75, pp. 679 – 689.
- Carvalho, F. C. (2010). Distribution and relative abundance of blue sharks in the Southwest Atlantic Ocean. Unpublished Master of Science thesis. University of Florida.
- Carvalho, F., Murie, D., Hazin, F., Hazin, H., Leite-Mourato, B., Travassos, P., and Burgess, G. (2010). Catch rates and size composition of blue sharks (*Prionace glauca*) caught by the Brazilian pelagic longline fleet in the southwestern Atlantic Ocean. *Aquatic Living Resource Journal*, 23, pp. 373 - 385.
- Chang, S-K., Lee, H-H. and Liu, H-I. (2007). Standardization of South Atlantic swordfish by-catch rate for Taiwanese longline fleet. *Collective Volume of Scientific Papers ICCAT*, 60, pp. 1974 – 1985.
- Chang, F-C., and Yeh, S-Y. (2014). Standardized CPUE of South Atlantic albacore (*Thunnus alalunga*) based on Taiwanese longline catch and effort statistics dating from 1967 to 2012. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1234 - 1246.
- Charrad M., Ghazzali N., Boiteau V. and Niknafs A. (2014). NbClust: an R package for determining the relevant number of clusters in a data set. *Journal of Statistical Software*, 61, pp. 1 - 36.
- Collette, B. B. (1995). *Xiphiidae. Peces espada. Guia FAO para Identificación de Especies para los Fines de la Pesca. Pacífico Centro-Oriental*. In: Fischer, W., Krupp, F., Schneider, W., Sommer, C., Carpenter, K.E. and Niem, V., eds. FAO, Rome. 3, pp. 1651 - 1652.
- Cooper, A. B. (2006). A guide to fisheries stock assessment. From data to recommendations. 1<sup>st</sup> ed. [pdf.] New Hampshire: NH Sea Grant College Program. Available at: [http://sedarweb.org/docs/page/stockassessmentguide\\_NHSeaGrant.pdf](http://sedarweb.org/docs/page/stockassessmentguide_NHSeaGrant.pdf) [Accessed 1 February 2016].

- Damalas, D., Megalofonou, P. and Apostolopoulou, M. (2007). Environmental, spatial, temporal and operational effects on swordfish (*Xiphias gladius*) catch rates of eastern Mediterranean Sea longline fisheries. *Fisheries Research*, 84, pp. 233 – 246.
- Deporte, N., Ulrich, C., Mahévas, S., Demanèche, S. and Bastardie, F. (2012). Regional métiers definition: a comparative investigation of statistical methods using a workflow applied to international otter trawl fisheries in the North Sea. *ICES Journal of Marine Science*, 69, pp. 331-342.
- Dunn, P. K. (2014). tweedie: Tweedie exponential family models. R package version 2.2.1.
- De Hoon, M. J. L., Imoto, S., Nolan, J., and Miyano, S. (2004). Open source clustering software. *Bioinformatics*, 20, pp. 1453 – 1454.
- Eisen, M. B., Spellman, P. T., Brown, P. O., and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. *Proceedings of the National Academy of Sciences USA*, 95, pp. 14863 – 14868.
- Gavaris, S. (1980). Use of a multiplicative model to estimate catch rate and effort from commercial data. *Canadian Journal of Fisheries and Aquatic Sciences*, 37, pp. 2272 – 2275.
- Govender, A., van der Elst, R. and James, N. (2003). Swordfish. Global lessons. 1<sup>st</sup> ed. [pdf] South Africa: WWF-South Africa. Available at: <http://hdl.handle.net/1834/921> [Accessed 1 February 2016].
- Grundlingh, M.L., (1983). On the course of the Agulhas Current. *South African Geographical Journal*, 65, pp. 49 – 57.
- Gulland, J.A., 1956. On the fishing effort in English demersal trawl fisheries. *Ministry of Agriculture, Fisheries and Food (United Kingdom)*(Serial 2), 20, pp. 1 – 41.
- Harley, S. J., Myers, R. A., and Dunn, A. (2001). A meta-analysis of the relationship between catch-per-unit-effort and abundance. *Canadian Journal of Fisheries and Aquatic Sciences*, 58, pp. 1705 - 1772.
- Hazin, H. G., Hazin, F., Travassos, P., Carvalho, F. C. and Erzini, K. (2007). Fishing strategy and target species of the Brazilian tuna longline fishery, from 1978 to 2005, inferred from cluster analysis. *Collective Volume of Scientific Papers ICCAT*, 60(6), pp. 2029 – 2038.
- Hazin, H. G., Hazin, F. H. V., Mourato, B., Carvalho, F. and Fredou, T. (2014). Standardized catch rates of swordfish (*Xiphias gladius*) caught by the Brazilian fleet (1978-2012) using generalized linear mixed models (GLMM) using delta log approach. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1875 - 1884.
- Hillborn, R. and Walters, C. J. (1992). *Quantitative Fisheries Stock Assessment. Choice, Dynamics and Uncertainty*. London: Chapman and Hall, p. 604.

- Hinton, M. G. and Maunder, M. N. (2003). Methods for standardizing CPUE and how to select among them. *Collective Volume of Scientific Papers ICCAT*, 56, pp. 169 – 177.
- Holdsworth, J.C., Sippel, T.J. and Saul, P.J. (2010). Movement of broadbill swordfish from New Zealand tagged with pop-up satellite archival tags. *New Zealand Fisheries Assessment Report 2010/4*, 28 p.
- Hoyle, S.D., Langley, A.D. and Campbell, R.A. (2014). Recommended approaches for standardizing CPUE data from pelagic fisheries. WCPFC-SC10-2014/ SA-IP-10.
- Ijima, H., Ochi, D., Nishida, T. and Okamoto, H. (2015). Standardization of CPUE for striped marlin (*Tetrapturus audax*) of Japanese longline fishery in the Indian Ocean. *IOTC-2015-WPB13-17*. [Accessed 1 February 2016].
- Itano, D. (2011). Life history, habitat and fisheries overview (University of Hawaii). U.S. West Coast swordfish workshop: working towards sustainability, May 10th – 11th, San Diego, California.
- Jennrich, R.I. and Sampson, P. F. (1976). Newton-Raphson and related algorithms for maximum likelihood variance component estimation. *Technometrics*, 18, pp. 11 – 17.
- Jørgensen, B. (1987). Exponential dispersion models (with discussion). *Journal of the Royal Statistical Society. Serial B*, 49, pp. 127 – 162.
- Kai, M. and Yokawa, K. (2014). CPUE standardization of the South Atlantic swordfish caught by Japanese longliners for 1990 – 2012. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1837 – 1842.
- Kimura, D.K. (1981). Standardized measures of relative abundance based on modelling log (c.p.u.e.), and the application to Pacific ocean perch (*Sebastes alutus*). *Journal of the Conseil International pour l'Exploration de la Mer*, 39, pp. 211 – 218.
- Kiyofuji, H. (2014). Standardized CPUE for North Atlantic albacore caught by the Japanese longline fishery. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1123 - 1138.
- Kolody, D., Davies, N., and Campbell, R. (2006). South-west Pacific swordfish stock status summary from multiple approaches. Stock Assessment Specialist Working Group paper WCPFC-SC2-2006/ SA WP-7. Western and Central Pacific Fisheries Commission, Manila, Philippines, 7 –18 August 2006. Available at: <http://icesjms.oxfordjournals.org/> [Accessed 1 February 2016].
- Kolody, D., Robinson, J. and Lucas, V. (2010). Swordfish catch rate standardization for the Seychelles semi-industrial and industrial longline fleets. *IOTC-2010-WPB-04*. [Accessed 1 February 2016].
- Kolody, D. (2011). Review of CPUE issues for the 2011 Indian Ocean swordfish stock assessment. [online]. 9<sup>th</sup> Working Party on Billfish, Indian Ocean Tuna Commission, Mahé, Seychelles. *IOTC-2011-WPB09-13*. Available at: <http://www.iotc.org/documents/review-cpue-issues-2011-io-swo-stock-assessment> [Accessed 1 February 2016].



- Lancia, R. A., Bishir, J. W., Conner, M. C. and Rosenberry, C. S. (1996). Use of catch-effort to estimate population size. *Wildlife Society Bulletin*, 24, pp. 731 – 737.
- Lauretta, M. V. and Walter, J. F. (2015). Standardized catch indices of skipjack tuna, *Katsuwonus pelamis*, from the United States pelagic longline observer program. *Collective Volume of Scientific Papers ICCAT*, 71, pp. 374 - 389.
- Lee, L-K., Hsu, C-C. and Chang, F-C. (2014). Albacore (*Thunnus alalunga*) CPUE trend from Indian core albacore areas based on Taiwanese longline catch and effort statistics dating from 1980 to 2013. *IOTC-2014-WPTmT05-19 Rev\_1*. [Accessed 1 February 2016].
- Lutjeharms, J. R. E. and Ansorge, I. J. (2001). The Agulhas return current. *Journal of Marine Systems*, 30, pp. 115 – 138.
- Lynch, P.D., Shertzer, K.W. and Latour, R.J. (2012). Performance of methods to estimate indices of abundance for highly migratory species. *Fisheries Research*, 125–126, pp. 27 – 39.
- MacNeil, M.A., Carlson, J.K. and Beerkircher, L.R. (2009). Shark depredation rates in pelagic longline fisheries: a case study from the Northwest Atlantic. *ICES Journal of Marine Science*. 66, pp. 1–12.
- MacQueen, J. (1967) Some methods for classification and analysis of multivariate observations. In: Le Cam, L. M. and J. Neyman, J. eds., *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability*, 1, pp. 281–297. Berkeley, CA: University of California Press.
- Marchal, P. M., Ulrich, C. M., Korsbrekke, K., Pastoors, M. A., Rackham, B. (2003). Annual trends in catchability and fish stock assessments. *Scientia Marina*, 67, pp. 63-73.
- Matsumoto, T. (2014). Standardized CPUE for South Atlantic albacore by the Japanese longline fishery. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1154 - 1168.
- Matsumoto, T., Ochi, D. and Satoh, K. (2015). Japanese longline CPUE for bigeye tuna in the Indian Ocean standardized by GLM. *IOTC-2015-WPTT17-34*. [Accessed 1 February 2016].
- Matsumoto, T. and Satoh, K. (2015). Japanese longline CPUE for yellowfin tuna (*Thunnus albacares*) in the Atlantic Ocean standardized using GLM up to 2013. *Collective Volume of Scientific Papers ICCAT*, 71, pp. 275 - 287.
- Maunder, M.N. and Starr, P.J. (2003). Fitting fisheries models to standardised CPUE abundance indices. *Fisheries Research*, 63, pp. 43 – 50.
- Maunder, M.N. and Punt, A. E. (2004). Standardizing catch and effort data: a review of recent approaches. *Fisheries Research*, 70, pp. 141 - 159.
- Mourato, B. L., Andrade, H. A., Amorim, A. F. and Arfelli, C. A. (2007). Standardized catch rate of swordfish (*Xiphias gladius*) caught by Santos longliners off southern Brazil (1971 – 2005). *Collective Volume of Scientific Papers ICCAT*, 60(6), pp. 1943 – 1952.

- Muths, D., Le Couls, S., Evano, H., Grewe, P., and Bourjea, J. (2013). Multi-genetic marker approach and spatio-temporal analysis suggest there is a single panmictic population of swordfish *Xiphias gladius* in the Indian Ocean. *PLoS ONE*, 8: e63558.
- Neilson, J. D., Smith, S., Royer, F., Paul, S. D., Porter, J. M. and Lutcavage, M. (2009). Investigations of horizontal movements of Atlantic swordfish using pop-up satellite archival tags. In: Nielsen, J. L., Arrizabalaga, H., Fragoso, N., Hobday, A., Lutcavage, M. and Sibert, J., ed., *Tagging and Tracking of Marine Animals with Electronic Devices*. Netherlands: Springer, pp. 145 – 159.
- Neilson, J.D. and Smith, S. (2010). Update on the Canadian program for pop-up satellite archival tagging of swordfish. *Collective Volume of Scientific Papers ICCAT*, 65, pp. 229 - 240.
- Nelder, J. A. and Wedderburn, R. W. M. (1972). Generalized Linear Models. *Journal of the Royal Statistical Society. Series A (General)*, 135, pp. 370 – 384.
- Nishida, T. and Wang, S. P. (2010). Estimation of the Abundance Index (AI) of swordfish (*Xiphias gladius*) in the Indian Ocean (IO) based on the fine scale catch and effort data in the Japanese tuna longline fisheries (1980-2008). *IOTC-2010-WPB-09*. [Accessed 1 February 2016].
- Nishida, T., Kitakado, T. and Wang, S-P. (2011). Estimation of the Abundance Index (AI) of swordfish (*Xiphias gladius*) in the Indian Ocean (IO) based on the fine scale catch and effort data of the Japanese tuna longline fisheries (1980 – 2010). *IOTC-2011-WPB09-14*. [Accessed 1 February 2016].
- Nishida, T. and Wang, S-P. (2014). CPUE standardization of swordfish (*Xiphias gladius*) exploited by Japanese tuna longline fisheries in the Indian Ocean using cluster analysis for targeting effect. *IOTC-2014-WPB12-21 Rev\_1*. [Accessed 1 February 2016].
- Ochi, D., Matsumoto, T., Nishida, T. and Kitakado, T. (2015). Update of standardized Japanese longline CPUE for yellowfin tuna in the Indian Ocean and consideration of standardization methods. *IOTC-2015-WPTT17-26*. [Accessed 1 June 2016].
- Okamoto, H. and Ijima, H. (2015). CPUE standardization of sailfish (*Istiophorus platypterus*) caught by Japanese longline fishery in the Indian Ocean from 1994 to 2014. *IOTC-2015-WPB13-26*. [Accessed 1 February 2016].
- Ortega-García, S. and Gómez-Muñoz, V. (1992). Standardization of fishing effort using principal components analysis: Tuna purse seiners. *Scientia Marina*, 56(1), pp. 17-20.
- Ortiz, M. (2007). Update of standardized catch rates by sex and age for swordfish (*Xiphias gladius*) from the U.S. longline fleet 1981-2005. *Collective Volume of Scientific Papers ICCAT*, 60(6), pp. 1994 - 2017.
- Pan, W. (2001). Akaike's information criterion in generalized estimating equations. *Biometrics*, 57, pp. 120 – 125.

- Pech, N., and Laloë, F. (1997). Use of principal component analysis with instrumental variables (PCAIV) to analyse fisheries catch data. *ICES Journal of Marine Science*, 54, pp. 32 - 47.
- Pelletier D. and Ferraris J. (2000). A multivariate approach for defining fishing tactics from commercial catch and effort data. *Canadian Journal of Fisheries and Aquatic Sciences*, 57, pp. 51 - 65.
- Podestá, G.P., Browder, J. A. and Hoey, J. J. (1993). Exploring the association between swordfish catch rates and thermal fronts on U.S. longline grounds in the western North Atlantic. *Continental Shelf Research*, 13, pp. 253 - 277.
- Poisson, F. (2009). Fate of the fish caught on longline gears and potential mitigation measures. *IOTC-2009-WPEB-15*. [Accessed 1 February 2016].
- Poisson, F. and Fauvel, C. (2009). Reproductive dynamics of swordfish (*Xiphias gladius*) in the southwestern Indian Ocean (Reunion Island). Part 2: fecundity and spawning pattern. *Aquatic Living Resources*, 22, pp. 59 – 68.
- Pons, M. and Domingo, A. (2014). Update of standardized CPUE of albacore tuna, *Thunnus alalunga*, caught by Uruguayan longliners in the Southwestern Atlantic Ocean (1983-2012). *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1007 - 1020.
- Pons, M., Forselledo, R. and Domingo, A. (2014). Standardized CPUE of swordfish (*Xiphias gladius*) caught by Uruguayan longliners in the Southwestern Atlantic Ocean (1982-2012). *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1758 - 1776.
- R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <https://www.R-project.org/> [Accessed 1 February 2016].
- Ramos-Cartelle, A., García-Cortés, B. and Mejuto, J. (2014). Standardized catch rates in biomass for the South Atlantic stock of swordfish (*Xiphias gladius*) from the Spanish longline fleet for the period 1989 – 2011. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1804 - 1811.
- Robson, D.S. (1966). Estimation of the relative fishing power of individual ships. *ICNAF Research Bulletin*, 3, pp. 5 – 14.
- Sandvik, B. (2013). Thematic mapping. [online]. Available at: <http://thematicmapping.org/> [Accessed 1 February 2016].
- Santos, M. N., Coelho, R. and Lino, P. G. (2013). An updated overview of the swordfish catches by the Portuguese pelagic longline fishery in the Indian Ocean between 1998-2012: catch, effort, CPUE and catch-at-size. *IOTC-2013-WPB11-31 Rev\_1*. [Accessed 1 February 2016].

- Santos, M. N., Coelho, R. and Lino, P. G. (2014a). Standardized CPUE for swordfish (*Xiphias gladius*) caught by the Portuguese pelagic longline fishery in the North Atlantic. *Collective Volume of Scientific Papers ICCAT*, 70(4), pp. 1783 – 1791.
- Santos, M. N., Coelho, R. and Lino, P. G. (2014b). Swordfish catches by the Portuguese pelagic longline sleet between 1998 – 2013 in the Southwest Indian Ocean: effort, standardized CPUE and catch-at-size. 12<sup>th</sup> Working Party on Billfish, Indian Ocean Tuna Commission, Yokohama, Kanagawa, Japan. *IOTC–2014–WPB12–19*. Available at: <http://www.iotc.org/sites/default/files/documents/2014/10/IOTC-2014-WPB12-19 - Portuguese SW-IO SWO LL CPUE.pdf> [Accessed 1 February 2016].
- Sedberry, G. R. and Loefer, J. K. (2001). Satellite telemetry tracking of swordfish, *Xiphias gladius*, off the eastern United States. *Marine Biology*, 139, pp. 355 – 360.
- Semba, Y., Nishida, T. and Wang, S-P. (2008). Preliminary results of standardization of swordfish CPUE of Taiwanese and Japanese tuna longline fisheries in the Indian Ocean (1980- 2006 and 1990-2006). 6<sup>th</sup> Working Party on Billfish, Indian Ocean Tuna Commission, Seychelles. *IOTC–2008–WPB03*. Available at: <http://www.iotc.org/sites/default/files/documents/proceedings/2008/wpb/IOTC-2008-WPB-03.pdf> [Accessed 1 February 2016].
- Shono, H. 2008. Application of the Tweedie distribution to zero-catch data in CPUE analysis. *Fisheries Research*, 93, pp. 154 – 162.
- Spierling, A.T., Neilson, J., Stone, H., and Carruthers, E. (2005). Compilation and analyses of Canadian conventional tagging data for swordfish (*Xiphias gladius*), 1961-2004. *Collective Volume of Scientific Papers ICCAT*, 58, pp. 1483 - 1494.
- Sun, C-L., Su, N-J. and Yeh, S-Z. (2014). Standardized CPUE of swordfish (*Xiphias gladius*) caught in the Taiwanese longline fishery in the South Atlantic Ocean for 1967-2012, addressing the targeting change. *Collective Volume of Scientific Papers ICCAT*, 70, pp. 1945 - 1953.
- Tascheri, R., Saavedra-Nievas, C. and Roa-Ureta, R. (2010). Statistical models to standardize catch rates in the multi-species trawl fishery for Patagonian grenadier (*Macruronus magellanicus*) off southern Chile. *Fisheries Research*, 105, pp. 200 – 214.
- Tibshirani, R. and Walther, G. (2005). Cluster validation by prediction strength. *Journal of Computational and Graphical Statistics*, 14, pp. 511 – 528.
- Tweedie, M.C.K. (1984). An index which distinguishes between some important exponential families. In: Ghosh, J.K., Roy, J. eds., *Statistics: Applications and New Directions*. Proceedings of the Indian Statistical Institute Golden Jubilee International Conference. Indian Statistical Institute, Calcutta, pp. 579–604.

- Venables, W. and Dichmont, C. (2004). GLMs, GAMs and GLMMs: an overview of theory for applications in fisheries research. *Fisheries Research* 70, pp. 319 – 337.
- Walter, J. (2015). Standardized catch rates for bigeye tuna (*Thunnus obesus*) from the pelagic longline fishery in the Northwest Atlantic and the Gulf of Mexico. *Collective Volume of Scientific Papers ICCAT*, 71), pp. 359 - 373.
- Walters, C.J. (2003). Folly and fantasy in the analysis of spatial catch rate data. *Canadian Journal of Fisheries and Aquatic Sciences*, 60, pp. 1433 – 1436.
- Wang, S-P. (2015). CPUE standardization of striped marlin (*Kajikia audax*) caught by Taiwanese longline fishery in the Indian Ocean using targeting effect derived from cluster and principle component analyses. *IOTC-2015-WPB13-31*. [Accessed 1 February 2016].
- Wang, S-P. and Nishida, T. (2013a). CPUE standardization of blue marlin (*Makaira mazara*) caught by Taiwanese longline fishery in the Indian Ocean. *IOTC-2013-WPB11-24 Rev\_2*. [Accessed 1 February 2016].
- Wang, S-P. and Nishida, T. (2013b). CPUE standardization of striped marlin (*Kajikia audax*) caught by Taiwanese longline fishery in the Indian Ocean. *IOTC-2013-WPB11-26 Rev\_2*. [Accessed 1 February 2016].
- Wang, S-P. and Nishida, T. (2014). CPUE standardization with targeting analysis for swordfish (*Xiphias gladius*) caught by Taiwanese longline fishery in the Indian Ocean. *IOTC-2014-WPB12-22 Rev\_1*. [Accessed 1 February 2016].
- Wedderburn, R.W.M. (1974). Quasi-likelihood functions, generalized linear models, and the Gauss–Newton method. *Biometrika*, 61, pp. 439 – 447.
- Winker H., Kerwath S. E., and Attwood C. G. (2013). Comparison of two approaches to standardize catch-per-unit-effort for targeting behaviour in a multispecies hand-line fishery. *Fisheries Research*, 139, pp. 118 - 131.
- Winker, H., Kerwath, S.E. and Attwood, C.G. (2014). Proof of concept for a novel procedure to standardize multispecies catch and effort data. *Fisheries Research*, 155, pp. 149 - 159.
- Yeh, Y-M. and Chang, S-T. (2013). CPUE standardizations for yellowfin tuna caught by Taiwanese longline fishery in the Indian ocean using Generalized Linear Model. *IOTC-2013-WPTT15-38*. [Accessed 1 February 2016].
- Yeh, Y-M. and Chang, S-T. (2015). Updated CPUE standardizations for bigeye and yellowfin tuna caught by Taiwanese longline fishery in the Indian Ocean using generalized linear model. *IOTC-2015-WPTT17-25*. [Accessed 1 June 2016].
- Yokawa, K. and Kai, M. (2014). Standardization of CPUE of swordfish caught by Japanese longliners in the North Atlantic. *Collective Volume of Scientific Papers ICCAT*, 70(4), pp. 1843 - 1856.

## CHAPTER 4

### Punchline: managing swordfish (*Xiphias gladius*) across overlapping tRFMO jurisdictions

#### *History of conventions and agreements on straddling stocks*

Regional fisheries management organisations (RFMOs) are international organisations formed by countries that have interests in the fishing in a region. The members can either be countries in the region, referred to as coastal states, or distant-water fishing nations (DFWNs). The RFMOs are responsible for conserving either a highly migratory species that span large geographical areas, such as the tunas and tuna-like species, or the species found in a particular geographical region. Tuna RFMOs (tRFMOs) differ from each other in their levels of responsibility and management, varying in focus species, data collection, research aims, management measures, incorporation of ecosystems approach to fisheries, compliance and enforcement of management measures.

The five tRFMOs include the Indian Ocean Tuna Commission (IOTC), International Commission for the Conservation of Atlantic Tuna (ICCAT), Commission for the Conservation of Southern Bluefin Tuna (CCSBT), Inter-American Tropical Tuna Commission (IATTC), and the Western and Central Pacific Fisheries Commission (WCPFC). The CCSBT manages one species in contrast to the other four tRFMOs that manage commercially important target species and bycatch within a defined convention area. Although there are geo-political boundaries separating these tRFMOs (Figure 1), the key species are highly migratory and on these scales boundaries are not finite entities adhered to by these species. The potential for highly migratory species to make transoceanic movements across boundaries requires multilateral cooperation and management among tRFMOs for best management of these valuable species.

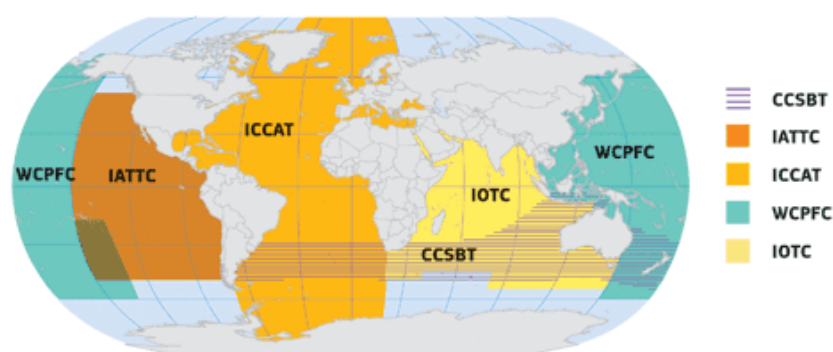


Figure 1. The boundaries separating the convention areas of the tuna RFMOs. Source: European Commission.

The 1982 United Nations Convention on the Law of the Sea (UNCLOS) marked a turning point in the human stewardship of the oceans: “States have the obligation to protect and preserve the marine environment” (Van Dyke, 1993, 2000). Words such as “protect”, “preserve” and “obligation” emphasised that countries must respect the natural processes of the ocean and must act in a manner that understands these processes and ensures that they continue for future generations (Allen *et al.*, 2010). Article 62 of UNCLOS addresses stocks that straddle adjacent exclusive economic zones (EEZs), or an EEZ and an adjacent high-seas area, and requires states to agree on measures to ensure the conservation of the species (Allen *et al.*, 2010). The 1995 Straddling and Highly Migratory Fish Stocks Agreement built on the 1982 United Nations Law of the Sea Convention, and strengthened the role of regional organisations and emphasised the need for coastal and island nations to cooperate with Distant Water Fishing Nations (DWFNs). The United Nations Fish Stocks Agreement (UNFSA) entered into force in 2001 and obliges both RFMO members and non-members that are parties to UNFSA to abide by the conservation and management measures adopted by the relevant RFMOs in order to have access to the fisheries (Allen *et al.*, 2010). The UNFSA places RFMOs in a pivotal and central position in terms of its implementation; they provide the primary mechanism through which States should cooperate to achieve enhanced resources conservation and management. Some RFMOs whose mandates extend to the conservation and management of straddling fish stocks and highly migratory fish stocks have reviewed, or are in the process of reviewing, certain provisions of their respective conventions to ensure that they are consistent with the Agreement. Other RFMOs, such as WCPFC and the South East Atlantic Fisheries Organisation (SEAFO), have spawned from the UNFSA and closely follows the structure of the agreement. It is under the basis and guidance of this Agreement that tRFMOs should start to manage straddling and highly migratory fish stocks.

The basic texts and agreements upon establishment of each tRFMO makes provision for future collaboration with other international fisheries commissions and scientific organisations, however the necessity to interact and co-manage has only been practiced through the establishment of overlapping areas. The CCSBT’s overlap with the three tRFMOs is less challenging since the IOTC, ICCAT and WCPFC relinquished the responsibility of managing and conserving southern bluefin tuna over its entire geographical range to the CCSBT. The WCPFC’s overlapping areas with the IATTC and IOTC have taken years to resolve, and the issues of vessel assignment, data reporting and implementing opposing management measures are yet to be finalised in the WCPFC/IOTC overlap. The WCPFC/IATTC overlapping area is purely a political zone that caters for the fishing interests of fleets from the western and eastern end of the French Polynesia region. Similarly the WCPFC/IOTC

overlap was for the WCPFC to cater for fishing interests of vessels in the Pacific Ocean that extended to that range. The ICCAT boundary in the South Atlantic Ocean ended at 20°E and the IOTC boundary in the South West Indian Ocean ended at 30°E until the boundary was modified in 1999 to 20°E to close the gap between the ICCAT and IOTC (IOTC, 1999).

#### *The nature of mixed stocks*

Studies on stock structure can inform the boundaries between stocks, and levels of mixing between stocks, ocean basins or RFMO boundaries. Stock assessments are the key approach to managing harvested fish species, and accurately defining the range and size of your stock is important for the estimates of biomass, spawner stock biomass (SSB), growth, mortality and recruitment of each stock and for the overall results of the assessment. Scientists have been conducting extensive research to understand and consider stock structure, species movement and degrees of mixing within and between the Atlantic Ocean, Mediterranean Sea, Indian Ocean, and the Pacific Ocean. When a stock exceeds the political boundaries or the predicted range of the stock, and neighbouring stocks of the same species mix boundaries, important factors to consider are to what degree are the stocks mixing, are the mixed stocks reproductively isolated or not and how is the catch accounted for in each of the neighbouring stock assessments. Fish stocks of a species can either be 1) one mixed stock that shows no reproductive isolation, 2) two mixing stocks that are reproductively isolated with spawning site fidelity, or 3) two separate stocks that show no mixing and are reproductively isolated. These three scenarios are referred to as “pooled”, “overlapping” and “separate” stocks, respectively, by the study of Li *et al.* (2015) on intermixing fish populations. A combination of studies such as tagging (conventional, satellite and genetic mark-recapture), size frequency, population genetics, otolith chemical analysis, organic components (stable isotopes such as carbon, nitrogen, fatty acids and proteins), chemical contaminants, parasite markers and reproductive biology analyses are required in a multifaceted holistic approach to gain the most accurate estimation of the stock structure (Begg and Waldman, 1999).

#### *The importance of management across RFMO jurisdictions*

When intermixing of fish stocks are not accounted for in stock assessments, it leads to the poor estimation of abundance and unintended depletion of local subunits (Hutchings, 1996, 2000; Stephenson, 1999; Frank and Brickman, 2000; Molton *et al.*, 2012, 2013). There are two main approaches to account for intermixing stocks in stock assessments; modify the spatial scale of fishery



assessments, such as pooling harvest among areas and assessing as a single stock which is the more common and basic approach, or the more complex approach would be to account for movement between stocks. The latter approach that seeks to account for stock mixing has been tested and is in future plans for implementation in species such as the Atlantic bluefin tuna (*Thunnus thynnus*). The *T. thynnus* stock assessment conducted by ICCAT will consider multi-stock assessment methods that include mixing (Taylor *et al.*, 2011).

## **Managing swordfish off South Africa: the punchline**

The population genetic results in chapter two of this thesis indicated that the swordfish stocks of the South Atlantic Ocean and the Indian Ocean coexist and are mixing around South Africa between (though not limited to) 14°E and 27°E. The political boundary that currently separates the convention areas of ICCAT and IOTC at 20°E has been used to divide South Africa's catch and effort data to report to the respective RFMO for the Atlantic and Indian oceans. Although a mixing ratio (Chow *et al.*, 2000) has not been calculated for this region, there is a portion of swordfish that originates from the neighbouring stock that ICCAT and IOTC are considering as their own. Furthermore, the ICCAT South Atlantic quota allocated to South Africa is being fulfilled with swordfish that originate from two different stocks. Accounting for this admixture zone has implications for (i) how South Africa's data is included in stock assessments, (ii) how the total catch is reported and (iii) how quotas are managed between ICCAT and IOTC, particularly when quotas and the status of the stocks differ vastly.

The results from chapter three indicated no qualitative difference in the trends of the abundance indices of models that included data from the admixed area with those that excluded it. This would address issue (i) and enable South Africa's data to be included in the stock assessments at the current 20°E boundary for the South Atlantic (<20°E) and the Indian Ocean (>20°E) stocks.

The tRFMOs should shift the boundary that separates the two swordfish stocks to 27°E for ICCAT and 14°E for IOTC (Figure 2). South Africa and the fleets fishing further offshore of the 200 nm EEZ should report catch and effort data for this transitional region to both tRFMOs (*i.e.* duplicate data will be held by ICCAT and IOTC for this region). These data will relate to calculations of fishing mortality and catch performance against quota allocations. Additionally, until a quantitative level of mixing is calculated, ICCAT and IOTC should consider that 50% of the catch in this transitional region is swordfish that has originated from their respective stocks.

The tRFMOs are progressively implementing the Management Strategy Evaluation (MSE) approach to meet the objectives of a fishery. MSE is a modelling based approach aimed at testing the robustness of possible management arrangements (plans) by examining which sets of decision rules, which are used to adjust TACs or effort controls, perform the best in achieving the management objectives for a fishery. This simulation testing can also be used to determine how robust the management plan is likely to be to uncertainties. These analyses enable the choice of which management planning option has the most reasonable likelihood of achieving the management goals (Butterworth and Punt, 1999; Holland, 2010). Further studies on spatial-temporal variations in the dynamics of swordfish (and other migratory stocks) population mixture between the Indian and Atlantic oceans have the potential to be incorporated in the MSE framework for ICCAT and IOTC.

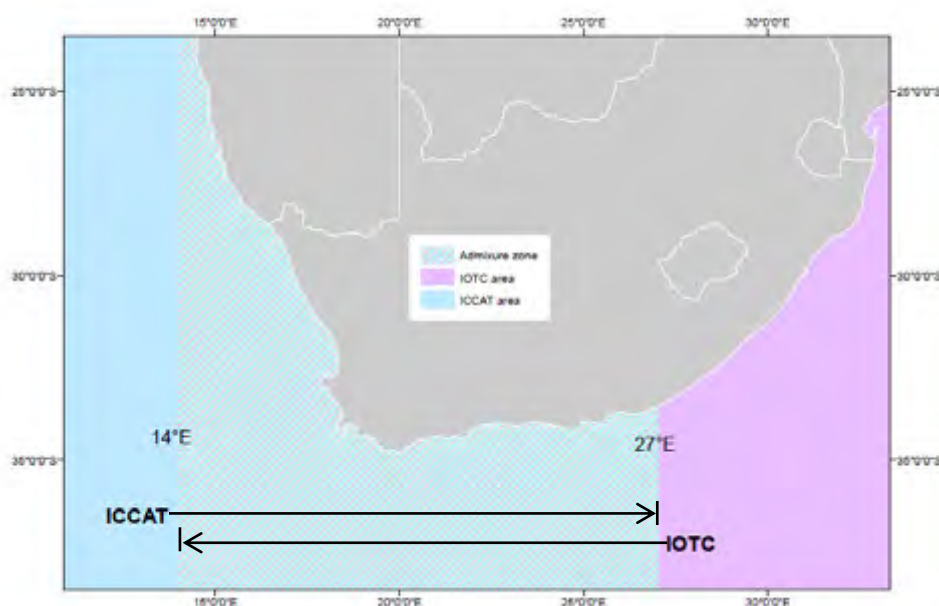


Figure 2. The recommended overlapping swordfish boundary of ICCAT and IOTC, including the admixture zone between 14°E and 27°E.

## References

Allen, R., Joseph, J. and Squires, D. (2010). *Conservation and Management of Transnational Tuna Fisheries*, edited by Allen, R., Joseph, J. and Squires, D. Ames, IA: Blackwell Publishing, pp. 155 – 179.

- Begg, G. A. and Waldman, J. R. (1999). An holistic approach to fish stock identification. *Fisheries Research*, 43, pp. 35 - 44.
- Butterworth, D. S. and Punt, A. E. (1999). Experiences in the evaluation and implementation of management procedures. *ICES Journal of Marine Science*, 56, pp. 985 – 998.
- Chow, S., Okamoto, H., Miyabe, N., Hiramatsu, K. and Barut, N. (2000). Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology*, 9, pp. 221 – 227.
- Frank, K. T., and Brickman, D. (2000). Allee effects and compensatory population dynamics within a stock complex. *Canadian Journal of Fisheries and Aquatic Sciences*, 57, pp. 513 – 517.
- Holland, D. S. (2010). Management strategy evaluation and management procedures: tools for rebuilding and sustaining fisheries. *OECD Food, Agriculture and Fisheries Working Papers*, No. 25, OECD Publishing, pp. 1 – 67.
- Hutchings, J. (1996). Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, pp. 943 – 962.
- Hutchings, J. A. (2000). Collapse and recovery of marine fishes. *Nature*, 406, pp. 882 – 885.
- IOTC (1999). Report of the Fourth Session of the Indian Ocean Tuna Commission. [online]. Kyoto, Japan: IOTC, p. ii. Available at:  
<http://www.iotc.org/sites/default/files/documents/proceedings/1999/s/IOTC-1999-S04-R%5BEN%5D.pdf> [Accessed 1 February 2016].
- Li, Y., Bence, J. R. and Brenden, T. O. (2015). An evaluation of alternative approaches for intermixing fish population: a case study with Great Lakes lake whitefish. *ICES Journal of Marine Science*, 72, pp. 70 – 81.
- Molton, K. J., Brenden, T. O., and Bence, J. R. (2012). Control rule performance for intermixing lake whitefish populations on the 1836 Treaty waters of the Great Lakes: a simulation-based evaluation. *Journal of Great Lakes Research*, 38, pp. 686 – 698.
- Molton, K., Brenden, T. O., and Bence, J. R. (2013). Harvest levels that conserve spawning biomass can provide larger and more stable and sustainable yields in intermixed fisheries. *Fisheries Research*, 147, pp. 264 – 283.
- Stephenson, R. L. (1999). Stock complexity in fisheries management: a perspective of emerging issue related to population sub-units. *Fisheries Research*, 43, pp. 247 – 249.
- Taylor, N. G., McAllister, M. K., Lawson, G. L., Carruthers, T. and Block, B. A. (2011). Atlantic bluefin tuna: a novel multistock spatial model for assessing population biomass. *PLoS ONE*, 6: e27693.

- Van Dyke, J. M. (1993). International governance and stewardship of the high seas and its resources. In: Van Dyke, J. M., Zaelke, D. and Hewison, G., ed., *Freedom for the Seas in the 21<sup>st</sup> Century*. Washington, DC: Island Press, pp. 13 – 22.
- Van Dyke, J. M. (2000). Sharing ocean resources in a time of scarcity and selfishness. In: Scheiber, H., ed., *The Law of the Sea: The Common Heritage and Emerging Challenges*. Publications on Ocean Development, Vol. 34. The Hague/London/Boston: Brill Academic Publishers, pp. 3 – 36.